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Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles?[1]

Louis Bernatchez and Pierre Duchesne

Abstract: We developed multivatiate analytical models to predict the probability of assigning offspring to parental couples as a function of population size, number of loci, and allelic diversity and the relationships between the probability of allocating individuals to their population of origin as a function of number of loci and allelic diversity. The parentage model predicts that the number of loci and number of alleles contribute interactively to increase assignment success. Given sufficient allelic diversity, a relatively low number of loci is required to achieve high allocation success even for relatively large numbers of possible parents. In contrast, the population model predicts an additive contribution of the number of loci and alleles. There appears to be no significant gain in increasing allelic diversity beyond approximately 6–10 alleles per locus in population assignment studies. Such predictive models should contribute to maximizing the returns of population and parentage assignment studies by increasing our understanding of interactions among the various variables affecting allocation success and by allowing the adjustment a priori of the required level of resolution and, consequently, optimizing the costs–benefits ratio in the use of molecular markers.

Résumé : Nous présentons ici deux modèles analytiques multivariés prédisant la probabilité d'assigner des rejetons aux couples parentaux en fonction du nombre de parents potentiels, du nombre de locus, de la diversité allélique et la relation entre la probabilité d'assigner des individus à leur population d'origine en fonction du nombre de locus et de la diversité allélique. Le modèle parental prédit une contribution interactive entre le nombre de locus et d'allèles sur l'accroissement du succès d'assignation. Étant donnée une diversité allélique suffisante, un nombre relativement faible de locus est requis pour atteindre un succès d'assignation élevé, même quand le nombre de parents possibles est grand. Inversement, le modèle populationnel prédit une contribution additive du nombre de locus et d'allèles. Peu de gain significatif est obtenu en augmentant la diversité allélique au-delà d'environ six à 10 allèles par locus dans les études d'assignation populationnelle. De tels modèles prédictifs devraient contribuer à maximiser le rendement d'études d'assignation parentale et populationnelle en améliorant notre compréhension des interactions entre les différentes variables pouvant influencer le succès d'allocation et en permettant d'ajuster a priori le niveau requis de résolution et donc, d'optimiser le rapport coût–bénéfice de l'utilisation des marqueurs moléculaires.

Introduction

The use of molecular genetics is increasingly contributing to our knowledge of fundamental issues in evolutionary biology of aquatic organisms. For instance, phylogeographic studies have been instrumental in elucidating patterns and processes of postglacial recolonization and highlighting the importance of historical events in shaping the genetic diversity of contemporary populations (Bowen and Avise 1995; Stanley et al. 1996; Colbourne et Hebert 1996; Bernatchez and Wilson 1998; Taylor and McPhail 1999). When used in combination with ecological approaches, the use of molecular genetics also contributed to our understanding of the role of evolutionary forces involved in population divergence and, ultimately, speciation events (e.g., Taylor and Bentzen 1993; Bernatchez et al. 1999; Lu and Bernatchez 1999; Turgeon et al. 1999). Such information, in turn, has been of paramount relevance for applied purposes, such as in defining evolutionary significant units for conservation (Dizon et al. 1992; Bernatchez 1995; Mayden 1995) and optimizing fisheries management (Utter and Ryman 1993; Carvalho and Hauser 1994; Ward and Grewe 1994).

Typically, most applications of molecular genetics rely on estimation of demographic parameters of diversity and differentiation that are derived from averaging the genetic composition over populations. It has been recognized for nearly 20 years, however, that further knowledge of relevance to both evolutionary biology and management may be obtained from the analysis of individual-based genotypic information (Foltz and Hoogland 1981; Hanken and Sherman 1981; Smouse et al. 1982). Yet, the potential of such applications remained relatively unexplored outside the studies of human and plant populations until recently (but see Jordan and Youngson 1992, and references therein). The most likely explanation for this is that the levels of accuracy and precision required in such studies were beyond the reach of available genetic markers (Smouse and Chevillon 1998). The blooming development of new genetic markers over the last de-

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cade, namely variable number of tandem repeat loci (VNTRs; microsatellites and minisatellites), has, however, revived a major interest in studies based on the definition of individual multilocus genotypes and opened exciting avenues of research and applications (reviewed in Estoup and Angers 1998; Davies et al. 1999).

Besides genetic mapping, studies based on the analysis of individual multilocus genotypes can be grouped into two broad categories of applications: parentage and population assignments. The former includes studies necessitating the assessment of precise parental relationships within populations, which may be achieved in various ways, including the use of exclusion probability, likelihood methods, and categorical and fractional parental assignment (reviewed in Marshall et al. 1998; see also Meagher and Thompson 1986). This may allow the defining of social structure (Amos et al. 1993), mating patterns (Clapham and Palsbøll 1997; Jones and Avise 1997), kinship (Fontaine and Dodson 1999), and quantification of reproductive success (Rico et al. 1992; Jones et al. 1998). Such analyses may also contribute improvement of the efficiency of selective breeding programs in domesticated populations (Herbinger et al. 1997; Estoup et al. 1998; Ferguson and Danzmann 1998). Studies of population assignments necessitate the determination of population membership of single individuals. This consists in assigning an individual to the population in which its multilocus genotype has the highest probability of occurring, assuming reliable allelic representation, Hardy–Weinberg equilibrium, and locus independence. Such estimation may be relevant to more precisely quantify gene flow and follow movements of individuals (Waser and Strobeck 1998; Palsbøll et al. 1997), determine the degree of differentiation among populations (Paetkau et al. 1995), and establish relationships among individuals within and among populations or higher taxonomic groupings (Nielsen et al. 1997; Roques et al. 1999). An extension of these aproaches is to detect the contribution of stocked fish in natural populations or to detect an admixture of populations in a sample of individuals of unknown origin (Banks et al. 1996; Nielsen et al. 1997; Tessier and Bernatchez 1998; Roques et al. 1999).

In contrast with the efforts made to find and develop markers suitable for individual-based multilocus genotype analyses, relatively little attention has been paid to determine how the characteristics of genetic markers may affect their usefulness for such purposes. Namely, it would be of particular interest to know a priori what combinations of numbers and types (especially in terms of allelic diversity) of loci should be used in order to reach a required level of resolution and optimize the use of such applications. The few studies that undertook such evaluation were based mainly on specific empirical observations or simulation studies derived from a finite set of loci with particular allelic distributions (e.g., Shriver et al. 1997; Estoup et al. 1998; Marshall et al. 1998). Such studies are certainly instructive; however, it remains unclear how much they can be generalized and used to make predictions in other contexts. This may be better achieved by a simulation exploration that allows a broader coverage of the vast spectrum of possible combinations of factors.

In this study, we used such an approach with the main objective of proposing multivariate analytical functions that could predict population and parentage assignment success as a function of number and properties of loci. For parentage analysis, we were interested in assessing the relationships between the probability of assigning offspring–parental couples in a close population as a function of population size, number of loci, and number and distribution of alleles per locus. We decided to focus on this particular issue for three reasons. First, this represents the most general situation of parentage analysis from which more specific cases can be derived. Second, exploration of parentage analyses at the population level and involving the identification of offspring–parental couples are scarce compared with the bulk of studies concerned with paternity analyses in which the mother–offspring is generally known (reviewed in Marshall et al. 1998). Such situations, however, apply only to those species providing parental care. In contrast, situations where both maternal and paternal parents, as well as their mating patterns, are unknown likely apply to a wider range of aquatic species, particularly in fishes. For population assignment, we were particularly interested in assessing the general relationships between the probability of allocating individuals to populations of origin, as well as its variance, as a function of number of locus and allelic diversity. We decided not to extend this investigation to the exploration of the effect of number of populations and their extent of divergence, which would justify a detailed analysis by itself. However, this has been partly investigated previously using a different procedure (Smouse et al. 1982). Although this paper focuses mainly on aquatic organisms, the models that we propose have general applicabilities and may be used for any sexually reproducing organisms.

Parentage assignment

We want to determine the probability Ps of successfully allocating an individual offspring to its parents based on its multilocus genotype. This is accomplished by the maximum likelihood method detailed in Sancristobal and Chevalet (1997). Briefly, this consists in computing the probability of occurrence of a given offspring genotype among the potential offsprings of each possible parental pair in a population. Once the probability of occurrence of the multilocus genotype of a given offspring is obtained, it is allocated to the parental couple showing the highest probability of producing it. We assume a Wright–Fisher type of reproduction and no prior knowledge on the sex of parents. The number of loci is N, the average number of alleles per locus is n, and the true parental pair belongs to a set of Ng potential parents. The Ps may be viewed as a random variable for a given combination of (n, N, Ng). Given this, we seek to obtain an analytical expression $P(N, n, \text{Ng})$ that will reflect the mathematical structure of relationships between Ps and these three variables. Next, we want to determine analytically, based on P, minimal conditions on the values of (N, n, Ng) so that Ps can be trusted to be at least 90% most of the time. Given any triplet (N, n, Ng), one would then be able to predict with confidence whether or not the 90% success rate threshold will be reached. The search for such minimal conditions led us to build P as a near lower bound for the distribution of Ps(n, N, Ng), thus representing a conservative modelling. We also

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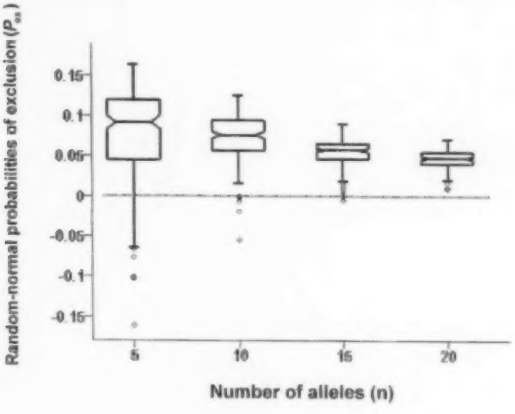
Fig. 1. Box plots representing empirical distributions of the differences in probability of exclusion P_{ex} between random allelic distributions and the normal allelic distribution associated with n = 5, 10, 15, and 20 alleles. For each number of alleles, 1000 random distributions were obtained by Monte-Carlo simulations using $U(0, 1)$. Almost all random distributions showed larger values of P_{ex} than the normal distribution for the same number of alleles. To obtain a normal allelic distribution for n (odd) alleles, we build the following list: $\{1/(n-1), 2/(n-2), \ldots, n/(n-1)\}$. Each number is then evaluated by the normal density function $N(1/2, 0.15)$. Finally, each member of this new list is divided by the sum of the list to get $\Sigma = 1$.



aimed at maintaining a small discrepancy (≈0.05) between $P(n, N, Ng)$ and the smallest Ps values.

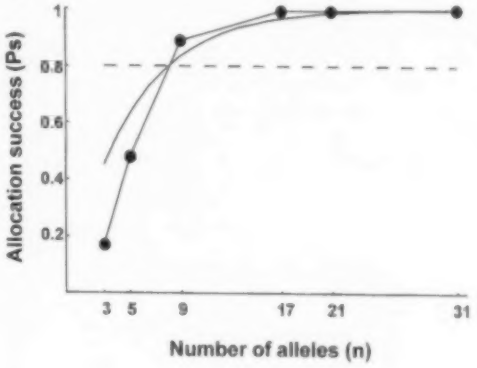
Methodological outlines

We first modellized the expectancy of Ps for allelic distributions with poor information content. To this end, a normal allelic distribution (detailed in the caption to Fig. 1) was constructed for any number of alleles per locus. The distribution of differences between the probability of exclusion P_{ex} (Smouse and Chakraborty 1986) obtained from random and normal allelic distributions of a same number of alleles illustrates the generally poor content of information of a normal allelic distribution for parentage analysis (Fig. 1). Thus, we expected that conservative modelling of the means of Ps for a normal distribution would lead to a reliable lower bound P for random allelic distributions.

To obtain an analytical expression $P(n, N, Ng)$ to be used as a conservative lower bound for the distribution of $Ps(n, N, Ng)$, we performed the following steps. First, simulations were done to collect estimates of the expectancy of Ps, $E(Ps)$, for each of a series of specific (N, n, Ng) combinations. We then proceeded to a conservative modelling of these estimates as an analytical expression, $P(N, n, Ng)$. Finally, the predictive potential of P as well as its structural similarity to Ps for random allelic distributions was tested by comparing the fit between independent data obtained by simulations and values predicted by the model.

Precisely, individual Ps values were computed with the following procedure: (1) construction of the normal allelic distribution for n alleles, (2) random generation of Ng parental genotypes for N loci with the allelic distributions obtained from step 1, (3) random generation of 100 offsprings from step 2, (4) parentage assignment of offsprings among all possible parent pairs using the maximum likelihood method, and (5) estimation of the proportion of correct allocations. The $E(Ps)$ values were estimated from the average of Ps values based on 20 such realizations for all combinations of the following parameter values: Ng = 10, 20, 30, 40, 50, and 70, N = 1, 2, 3, 4, 5, 6, and 7, and n = 3, 5, 9, 17, 21, and 31. All procedures were performed using programs written with the algebric computer system Maple V, version 5. A detailed description of the procedure used for estimating parentage assignment of offsprings is provided in Appendix 2.

Fig. 2. Observed relationship between estimates of the expectancy of allocation success Ps and number of alleles n together with the corresponding analytical curve in the case of five loci and 20 parents.



Stepwise modelling of $E(Ps)$ for normal allelic distributions

The modelling of $E(Ps)$ as a function of n, N, and Ng was done in four steps. We first established the relationship between the number of alleles n and $E(Ps)$. The empirical data show that $E(Ps)$ is an increasing function of n that converges to unity (Fig. 2). The rate of increase of $E(Ps)$ is regularly diminishing with n. A simple, straightforward way of modelling such behaviour is

$$(1) \qquad p(n) = 1 - (1/s)^n.$$

Because we were mainly interested in high values of $E(Ps)$, we choose s so that $p(n_0) = 0.8 = Ps(n_0)$. In other words, the desired s makes the empirical and analytical curves cross precisely when they reach 0.8 (Fig. 2). Using this procedure, we computed s for each number of loci (N = 1–7). As exemplified in Fig. 2 for N = 5, $p(n)$ remained under the empirical data for all values over 0.8, in agreement with our search for a lower bound for Ps. The same pattern was observed for other numbers of loci.

The speed parameter s is dependent on N and consequently may be viewed as a function of the number of loci. The second step was thus to modellize the functional relationships between the number of loci and s. The empirical relationship between s and N (varying from 1 to 7) has an obvious linear component (Fig. 3). The number of parents Ng clearly influences $s(N)$ (Fig. 3). For each of Ng = 10, 20, 30, 40, 50, and 70, we fitted the parameters m and b to their

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Fig. 3. Empirical relationships between the number of loci N and the speed parameter s as defined in eq. 1 for various numbers of parents (10, 20, 30, 40, 50, and 70).

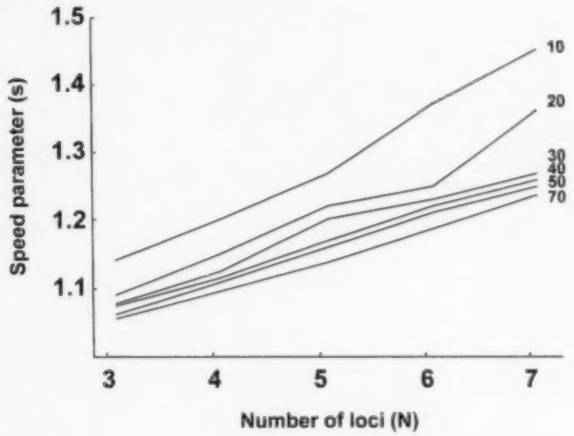
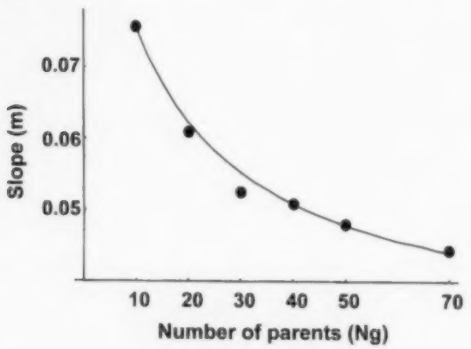


Fig. 4. Empirical relationship between the number of parents Ng and the slope parameter m as defined in eq. 2 along with the fitted analytical curve.



respective empirical $s(N)$ curves. In all cases, b was estimated at ≈ 0.915 such that

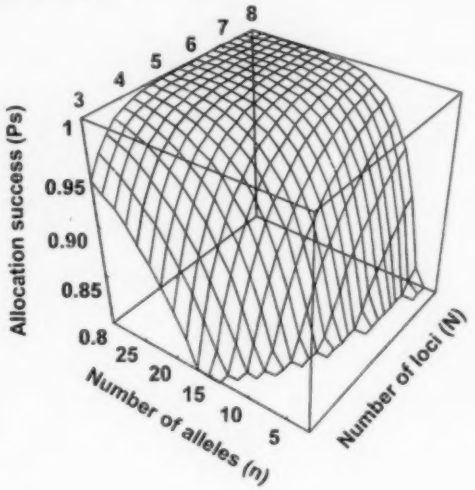
$$(2) \qquad s(N) = mN + 0.915.$$

The third step was thus to modellize the functional relationships between the number of parents Ng and m. The empirical relationships between m and Ng (varying from 10 to 70) show a decreasing trend in the form $1/x$. A satisfactory fitting between empirical data and the model (Fig. 4) was reached with the following equation:

$$(3) \qquad m(\mathrm{Ng}) = \frac{1}{(\mathrm{Ng} + 13)} + 0.032.$$

Equating eqs. 1, 2, and 3, we now define $P(n, N, \mathrm{Ng})$, the relationships between parentage assignment success as a function of population size (Ng), number of loci (N), and number of alleles per locus (n), as follows:

Fig. 5. According to the analytical model P, the surface representing the functional relationship between allocation success P, number of loci N, and average number of alleles n per locus in the case of 20 parents.



$$(4) \qquad P(n, N, \mathrm{Ng}) = 1 - \left(\frac{1}{\left(\frac{1}{\mathrm{Ng} + 13} + 0.032 \right) N + 0.915} \right)^n.$$

Figure 5 illustrates the relationships between the predicted parentage assignment success P, n, and N for Ng = 20. The graph illustrates that for any given number of loci, it is always possible to tend towards $P = 1$ by increasing the number of alleles per locus. Clearly, this indicates that increasing the number of alleles is highly advantageous in parentage assignment. Also, the rate at which P approaches unity depends strongly on the number of loci. Equation 4 also predicts that parentage assignment success will decrease with an increasing number of parents, but not linearly (see below).

Modelling E(Ps) for uniform allelic distributions

In order to explore the generality of predicting parentage assignment from a simple analytical function, we use the stepwise procedures described above to model E(Ps) as a function of n, N, and Ng for uniform allelic distributions (identical frequencies of $1/n$ for all alleles). A modelling procedure identical to the one applied for a normal allelic distribution led to the following analogous expression:

$$P_{\mathrm{uni}}(n, N, \mathrm{Ng}) = 1 - \left(\frac{1}{\left(\frac{1}{\mathrm{Ng} + 6} + 0.057 \right) N + 0.88} \right)^n.$$

which reflects a mathematical structure identical to that obtained for a normal allelic distribution. Given that P_{ex} values

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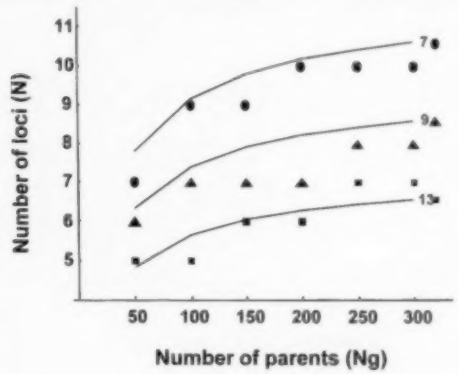
Fig. 6. Empirical points and analytical curves representing minimal number of loci N to reach allocation success Ps ≥ 0.90 as a function of number of parents Ng for 7, 11, and 13 alleles.



obtained for most randomly generated allelic distributions stand between P_{norm} and P_{uni}, their structural similarity may indicate the generality of the relationships between parentage assignment success (Ps) as a function of (N, n, Ng).

Predictive potential

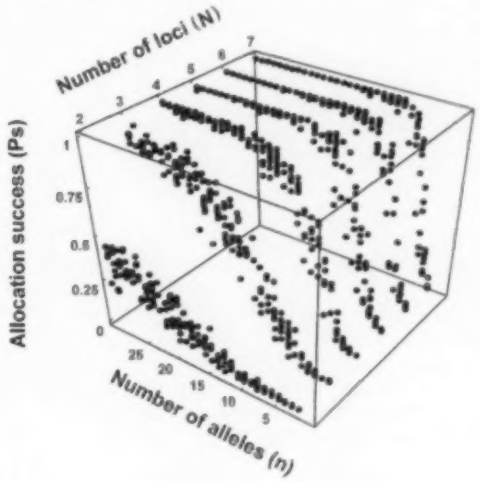
As stated above, our goal was to identify (N, n, Ng) triplets for which Ps will be at least 0.90. Thus, we empirically searched for the minimal N values that, given a normal allelic distribution and specific values of n and Ng, were large enough to ensure that Ps ≥ 0.90. This quantity was denoted $N90$. Simulations were done to estimate $N90$ in all combinations of the following number of alleles (n = 7, 9, 11, and 13) and number of parents (Ng = 50, 100, 150, 250, and 300). Each estimate of $N90$ resulted from the random generation of parents and 100 offspring as described above. Then, we compared these empirical $N90$ values with analytical N derived from $P(N, n, \text{Ng})$. Analytical N was evaluated as an explicit function of (n, Ng, P) derived from eq. 4:

$$(5)\quad N_{norm}(n, \text{Ng}, P) = \frac{0.125\left(1000\left(\frac{1}{1-P}\right)^{\frac{1}{n}}\text{Ng} - 915\text{Ng} + 13\,000\left(\frac{1}{1-P}\right)^{\frac{1}{n}} - 11895\right)}{(177 + 4\text{Ng})}$$

We found that N evaluated at P = 0.9 generally exceeded $N90$ for any combination of n and Ng. We reasoned that if the conservative nature of eq. 4 was the main source of discrepancy, then we should be able to find a smaller value for P such that $N90$ and N would agree most of the time. In fact, we found that P = 0.83 produced a very close approximation to empirical $N90$ (Fig. 6). In other words, given specific numbers of alleles and number of parents, if one sets $N = N_{norm}(n, \text{Ng}, 0.83)$, then the triplet ($N$, n, Ng) will be minimal in the sense that an increase in either N or n or a decrease in Ng will only raise its allocation power and consequently the confidence that Ps > 90%.

In order to further investigate the generality of the above equation to assess P as a lower bound for individual realisations of Ps, we performed simulations with random allelic distributions involving random sets of 20, 70, and 100 parents. For each N loci varying from 2 to 7 (from 2 to 8 in the case of Ng = 100), 200 n values (restricted between 2 and 31) were randomly generated. One hundred offspring were also randomly generated for each combination of (N, n, Ng), from which the proportion of correct allocations (Ps) was computed. It can be seen from Fig. 7 that P and the random realizations of Ps have very similar structures. We then divided the empirical triplets (N, n, Ng) into two categories: those with equal or greater power than minimal triplets and those with lesser power than minimal triplets. For both categories and each number of loci, we computed the proportion of realizations having Ps ≥ 0.90. In the high-power category, we found very high proportions of Ps ≥ 0.9 for all numbers of loci except N = 2 and N = 3. The overall percentage of high values was 84% for Ng = 20, 98% for Ng = 70, and 100% for Ng = 100. Excluding triplets with N = 2 or 3, we obtained 95% for Ng = 20, 100% for Ng = 70, and 100% for Ng = 100. Conversely, the low-power category yielded low percentages of high values (Ps ≥ 0.90) for Ng = 20 (7%), Ng = 70 (10%), and Ng = 100 (12%). Because the procedure to determine analytical minimal triplets was based on data with Ng as high as 300, we believe that similar results would be obtained for higher numbers of parents as well.

Fig. 7. Three-dimensional representation of 1200 triplets (N, n, Ps), each composed of a number of loci N chosen at random from 2 to 7, a number of alleles n chosen at random from 2 to 31, and of the allocation success Ps resulting from a Monte-Carlo simulation in the case of 70 parents.



Practical use of minimal triplets (N, n, Ng)

Minimal triplets may be used to determine the minimal number of loci to reach a parental assignment success $P \geq 0.90$. For instance, if the number of parents is 200 and the average number of alleles per locus is 7, then a minimal N would be $N_{norm}(7, 200, 0.83) \approx 10$. Conversely, one may seek a minimal number of alleles per locus, given an upper limit on the number of loci. Take the case of N = 6 and Ng = 250. Using eq. 5, one finds four minimal triplets satisfying these initial conditions: (6, 13, 250), (6, 14, 250), (6, 15, 250), and

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initial conditions: (6, 13, 250), (6, 14, 250), (6, 15, 250), and (6, 16, 250). Similarly, it can be verified that given 9 loci and 8 alleles per locus, any triplet with a number of parents in the range of 150–300 parents will be minimal. Because such calculations may be tedious, we programmed an electronic spreadsheet to compute minimal triplets over a user-defined array of numbers of parents. Appendix 1 provides a table of selected output values. This spreadsheet is available at the following internet address: http://www.bio.ulaval.ca/LBernatchez.html. Note that P should be viewed as a preanalysis predictor. A more accurate after-analysis estimate of Ps should be sought by running simulations with the established genetic data.

Population allocation

We want to determine the probability Ps of successfully allocating an individual to its population of origin based on its multilocus genotype, and population-specific allelic distribution, which is accomplished by a maximum likelihood algorithm (Shriver et al. 1997). This consists in assigning an individual to the population in which its multilocus genotype has the highest probability of occurring, assuming reliable allelic representation, Hardy–Weinberg equilibrium, and locus independence. Once the probability of occurrence of the multilocus genotype of a given offspring is obtained, it is allocated to the population showing the highest probability of producing it. The Ps will depend on the number of loci N and the average number of alleles per locus n. Given N and n, and considering the distribution of Ps over all possible pairs of allelic distributions between two populations, Ps may be viewed as a random variable. Given this, we first want to explore the relationships of the distribution of Ps as a function of number of loci and average number of alleles per locus with a random allelic distribution. We then seek a bivariate analytical expression $P(N, n)$ that will predict the behaviour of the expectancy of Ps as a function of number of loci and alleles, given all possible allelic distributions.

Methodological outlines

We first estimate the means of Ps for a number of pairs (N, n), which serve as a basis for modelling $P(N, n)$. Then, P is validated through Monte-Carlo simulations with values of (N, n) lying outside the initial modelling domain. Finally, we investigate the precision of $P(N, n)$ as a predictor of individual realisations of Ps for given values of N and n. Precisely, individual Ps values were computed for a given combination of (N, n) using the following procedure: (1) generation of two random allelic distributions, (2) construction of all possible genotypes for each allelic distribution obtained in step 1, and (3) computation of the proportion of successful allocations from step 2. Note that given a specific pair of allelic distributions, the value of Ps obtained is exact and does not depend on a random production of genotypes (Roques et al. 1999). Thus, the probability of correct allocation for any given multilocus genotype g can be exactly computed according to the formula

$$p(g) = \frac{\mathrm{Max}\,(p_1(g), p_2(g))}{p_1(g) + p_2(g)}$$

where $p_1(g)$ is the probability of genotype g in population 1 and $p_2(g)$ is the probability of genotype g in population 2. The expected probability of allocation success over all genotypes is exactly equal to the weighted sum of probabilities:

$$P_G = \sum_{g \in G} p(g)\left(\frac{1}{2}\,p_1(g) + \frac{1}{2}\,p_2(g)\right)$$

To exhaustively run through all possible multilocus genotypes, we first generated this set symbolically. The $p_1(g)$ and $p_2(g)$ values were then computed by substitution of allelic frequencies for the allele symbols. Besides providing precision, this approach proved to be fast enough to generate a large number of pairs of allelic distributions for each combination (N, n). Thus, the expectancy of Ps, E(Ps), was estimated from the average of 1500 pairs of allelic distributions for each of the following combinations: 1 locus, 2–28 alleles; 2 loci, 2–10 alleles; 3 loci, 2–6 alleles; 4 loci, 2–4 alleles; 5 loci, 2 and 3 alleles; 6 loci, 2 alleles; 7 loci, 2 alleles. Increasing the number of combinations was limited by the explosive increment in number of possible multilocus genotypes with increasing number of loci and alleles. All of these procedures were performed using programs written with the algebric computer system Maple V, version 5.

Stepwise modelling of E(Ps)

The modelling of E(Ps) as a function of n and N was done in three steps. We first established the relationships between the number of alleles n and E(Ps). For each number of loci N, E(Ps) is an increasing function $p(n)$ of the number of alleles, which is bounded by an asymptotic value α_N smaller than 1 (Fig. 8, top panel), that is dependent on the number of loci. These properties, combined with data compatibility, led to the following analytical expression:

$$(6) \qquad p_N(n) = \alpha_N - \frac{1}{2}^{n+2} \quad .$$

Second, we modellized the relationship between the number of loci and E(Ps) for $n = 2$. The empirical data indicate that the function $\tilde{p}_2(N)$ has an asymptotic value of 1 (Fig. 8, bottom panel). Moreover, since it is increasing monotonically, a suitable analytical model is $1 - \left(\frac{1}{a}\right)^{N+b}$, $a > 1$. A satisfactory fitting for N ranging from 1 to 8 (Fig. 8, bottom panel) was reached for $a = 1.25$ and $b = 4$ such that

$$(7) \qquad \tilde{p}_2(N) = 1 - \frac{4}{5}^{N+4} \quad .$$

Third, we established the relationships between E(Ps) and pairs of (N, n). If α_N is quantified, then eq. 6 can provide an explicit bivariate function $P(N, n)$. This can be solved by equating eqs. 6 and 7, which have to be equal at $n = 2$:

$$\alpha_N = \frac{17}{16} - \left(\frac{4}{5}\right)^{N+4} \quad .$$

which is then substituted into eq. 6 to define $P(N, n)$, the population assignment success as a function of number of loci and number of alleles per locus, as follows:

$$p(N, n) = \frac{17}{16} - \left(\frac{4}{5}\right)^{N+4} - \left(\frac{1}{2}\right)^{n+2} \quad .$$

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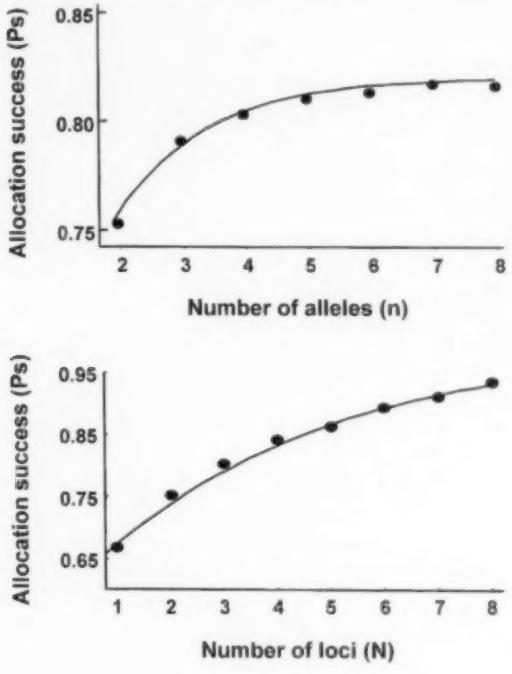
Fig. 8. Top panel: empirical data and analytical curve describing the relationship between the expectancy of allocation success Ps and the number of alleles n in the case of two loci. Bottom panel: empirical data and analytical curve describing the relationship between Ps and the number of loci N in the case of two alleles.



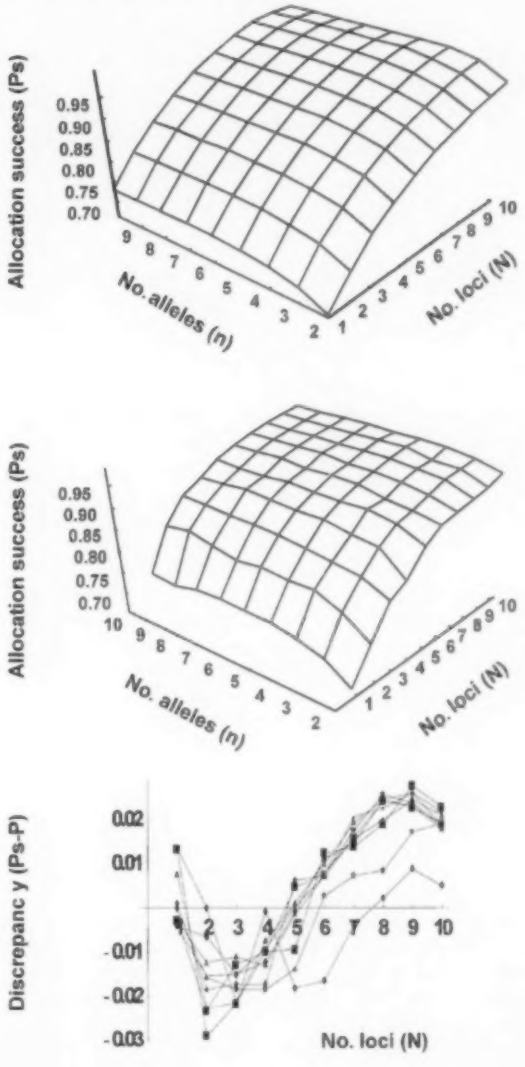
Because the least upper bound of $P(N, n)$, 17/16, is slightly larger than unity, we make a minor correction to the above expression to generate the final model:

$$(8) \qquad P(N,n) = \mathrm{Min}\left(1, \frac{17}{16} - \left(\frac{4}{5}\right)^{N+4} - \left(\frac{1}{2}\right)^{n+2}\right).$$

One important feature of $P(N, n)$ is the additive nature of the respective contributions of number of loci and number of alleles. In other words, according to the proposed model, there is no interaction between N and n. Figure 9 (top panel) illustrates the relationships between the predicted population assignment success P, n, and N. It can be seen that P increases rapidly as a function of N and that it will always be possible to nearly reach $P = 1$ by increasing N for any value of n. Given a moderate average number of alleles per locus, the expected minimal number of loci to reach high levels of allocation success ($P > 0.90$) between two populations is not overwhelming. In contrast, P is relatively independent of the average number of alleles per locus. Thus, for any number of loci, P first increases by augmenting the number of alleles per locus but rapidly reaches an asymptot at approximately $n = 6$. Recall that for any fixed number of loci N, the asymptotic value of P is

$$\alpha_N = \frac{17}{16} - \left(\frac{4}{5}\right)^{N+4}.$$

Fig. 9. Top panel: analytical model $P(N, n)$ shown as a surface over the domain (N = 1–10) × (n = 2–10) of values of number of loci N and number of alleles n. Middle panel: three-dimensional grid structure representing estimates obtained from Monte-Carlo simulations of expectancies of allocation success Ps over the domain (N = 1–10) × (n = 2–10) of values of N and n. Bottom panel: family of curves showing the discrepancies y between Ps and analytical values obtained from the model $P(N, n)$. Each curve traces $E(\mathrm{Ps}) - P(N, n)$ as a function of N given a specific n ranging from 2 to 10.



Hence, the maximum gain in P from increasing the number of alleles is $\alpha_N - P(N, 2)$, or

$$\left(\frac{17}{16} - \frac{4}{5}^{N+4}\right) - \left(\frac{17}{16} - \frac{4}{5}^{N+4} - \frac{1}{2}^{2+2}\right) = \left(\frac{1}{2}\right)^4 \approx 0.06$$

for any N value. This maximum gain is reached as soon as $n = 6$ since

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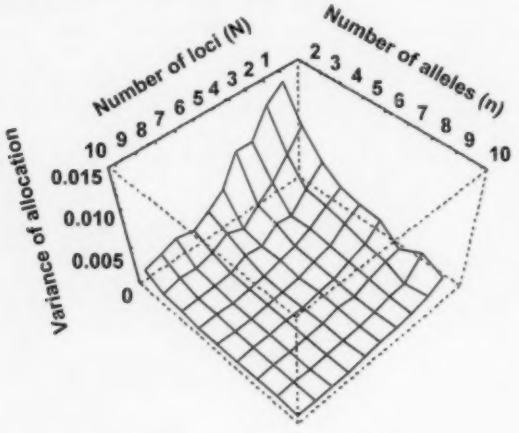
Fig. 10. Three-dimensional grid structure representing estimates obtained from Monte-Carlo simulations of variances of allocation success over the domain (N = 1–10) × (n = 2–10) of values of number of loci N and number of alleles n.



$$\left(\frac{1}{2}\right)^{2+2} - \left(\frac{1}{2}\right)^{6+2} \approx 0.06.$$

Clearly, increasing the number of alleles per locus beyond this approximate number of alleles adds little to population assignment success.

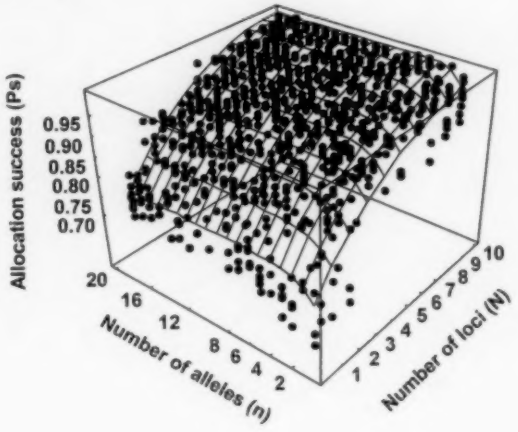
Validation of $P(N, n)$

In order to validate $P(N, n)$, estimates of E(Ps) were calculated for all possible pairs (N, n) for N varying between 1 and 10 and n varying between 2 and 10. For each pair, we performed 100 iterations of the following Monte-Carlo simulation procedure: (1) production of a pair of random allelic distributions, (2) random generation of 500 specimens for each population with allelic distribution generated in step 1, and (3) computation of the proportion of allocation success Ps. For each (N, n), E(Ps) was estimated from the average of the 100 individual Ps values. Note that the above procedure is distinct from the one previously described to generate the model. The main distinctive feature is the exactness of the former, due to the systematic generation of the whole set of genotypes rather than a partial random set. Figure 9 (middle panel) illustrates the strong structural similarity between the three-dimensional web of estimates of E(Ps) and the predicted surface $P(N, n)$. The discrepancy between both estimates is shown in Fig. 9 (bottom panel). The $P(N, n)$ underestimates E(Ps) for smaller numbers of loci ($N < 6$) whereas E(Ps) is overestimated for $N \geq 6$. The discrepancy curves for various values of n are very similar. Hence, the discrepancy appears to stem essentially from the number of loci N. Globally, however, the fit between the model and validation data was excellent, since the maximum absolute difference in allocation success between both methods was less than 0.03.

Predictive potential of $P(N, n)$

So far, we have been focusing on the mean values of Ps distributions to generate and validate the predictive model.

Fig. 11. Three-dimensional representation of 1000 triplets (N, n, Ps), each composed of a number of loci N chosen at random from 1 to 10, a number of alleles n chosen at random from 2 to 20, and of the allocation success Ps resulting from a Monte-Carlo simulation.



To assess the potential of $P(N, n)$ for predicting random values of Ps, however, we quantified the dispersion of the Ps distributions. The data that served to compute mean Ps estimates in the validation procedure also served to estimate variances of Ps (Fig. 10). The dispersion of Ps decreases rapidly as a function of increasing values for both N and n. Hence, Ps distributions with high mean values will have lower dispersions, and consequently, higher values of P will tend to be better predictors. This also indicates that while increasing the number of alleles per locus has little effect on increasing Ps, this may contribute to lowering the discrepancy between predicted and observed values of assignment success.

To evaluate specifically the predictive power of P, we ran additional simulations to produce 1000 individual values of Ps that were generated as follows: (1) random generation of N loci, varying between 1 and 10, (2) random generation of n alleles, varying between 2 and 20, (3) production of a pair of allelic distributions with N loci and n alleles per locus, (4) production of 500 random genotypes for each of both allelic distributions from step 3, and (5) computation of the proportion of allocation success Ps.

The distribution of the 1000 triplets (N, n, Ps) thus obtained is illustrated in Fig. 11. They show high similarity with the model $P(N, n)$. The predictive power of P can be quantified as the percentage of Ps values lying inside the interval $(P - \varepsilon, P + \varepsilon)$, where ε is a discrepancy value. This showed that Ps has a probability of at least 0.80 of lying inside $(P - 0.05, P + 0.05)$ for any value of P over 0.85. That is, P predicts Ps ± 0.05 whenever P is relatively high. Note that the 0.05 level compounds the error due to the model and the inherent variability of Ps.

Practical use of $P(N, n)$

Suppose one wants to estimate the minimum number of loci that is necessary to ensure high level of allocation success. First, we solve $P(N, n) = P$ for N from eq. 3:

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$$N(n, P) = \frac{12\ln(2) - 4\ln(5) - \ln(17 - 4 \cdot 2^{(-n)} - 16P)}{\ln(5) - 2\ln(2)}.$$

As an example, take an average number of alleles $n = 6$ and a desired level of success Ps = 0.90. Taking into account the error level 0.05, a conservative strategy is to aim at Ps = 0.90 + 0.05 = 0.95. An estimated minimal number of loci is $N(6, 0.95) \approx 6$. One may also be interested in the minimal number of alleles per locus n, given some upper limit on the number of loci. For instance, given $N = 7$, using the above technique and a few trial-and-error evaluations of $N(n, P)$, one finds that $n = 3$ is sufficient to reach Ps ≥ 0.90 in the majority of cases. We programmed an electronic spreadsheet to compute minimal triplets (N, n, Ps) over user-defined arrays of values of n and desired Ps. This spreadsheet is available at the following internet address: http://www.bio.ulaval.ca/LBernatchez.html. As for parental allocation, P should be viewed as a preanalysis predictor. A more accurate after-analysis estimate of Ps should be sought by running simulations with the established genetic data.

Discussion

Our main objective was to use a simulation approach in order to develop multivatiate analytical functions that could predict population and parentage assignment success as a function of number and properties of loci. In both cases, we were able to generate a model with a relatively high predictive power over a wide range of possible situations. Previous studies have described similar trends in relationships between the various parameters that we used in this study. In parentage assignment, for instance, Estoup et al. (1998) observed an increase in allocation success as a function of number of loci and their probability of exclusion, which is highly correlated with allelic diversity. In this study, allocation success also decreased with an increase in the possible number of matings. Similar trends were also reported by Marshall et al. (1998) for paternity analysis. The conclusions of these studies, however, were restricted to observations made from simulations derived from a restricted set of loci with specific allelic distribution. For population assignment studies, Smouse and Chevillon (1998) also reported an increase in allocation success with an increasing number of loci, although they remained relatively vague on the effect of numbers of alleles per locus. Furthermore, none of these studies attempted to explore the multavariate effects of various parameters (e.g., allocation success as a function of number of loci and numbers of alleles per locus). As such, the present study represents to our knowledge a first attempt to generate more general models that can be used to explore the interactive effects of various parameters on allocation success and also to predict allocation success over a wide range of conditions, in both parentage and population assignment studies.

For parentage assignment, the analytical model was built so as to reflect the structure of relationships involving the proportion of successful parental allocation Ps and the triplet of variables (N, n, Ng). In situations where these parameters can be evaluated at least approximately before undertaking a given study, the predictions derived from the model may be used to decide on a minimal number of loci or average number of alleles per locus to be used to reach a satisfactory level of allocation success. Ideally, it would have been desirable to take into consideration finer genetic information, such as specific allelic distribution. In our view, however, this can hardly be incorporated in preanalysis decision making processes. Consequently, our strategy was to develop a model specifically from normal allelic distributions. Despite their poor information content, these showed high structural homology with the whole set of possible allelic distributions. This means that our model may serve as a general approach for predicting the functional relationships between Ps and (N, n, Ng) as well as a lower bound for Ps for all possible allelic distributions.

A first important prediction of the model was that for any number of loci, one can always increase the average number of alleles to reach Ps ≈ 1. The model also predicts that N increases the rate with which n is acting on Ps. Hence, the effects of n and N are closely linked. A second prediction is that the number of parents in the population exerts a dampening effect over the number of loci, but this effect decreases with increasing Ng. Practically, this means that the addition of an extra locus compensates for ever larger increases in the number of parents, such that a reasonable number is required to achieve high allocation success even for relatively high numbers of possible parents, given sufficient allelic diversity. For instance, seven loci with an average number of alleles of 13 would be required to reach an allocation success of 0.90 in a population of 300 possible parents (Appendix 1). There are few empirical data available at this time that could be compared with the predictions of our model. In an ongoing study of parentage assignment in Atlantic salmon (*Salmo salar*), we found that six loci were sufficient to reach an allocation success of $P = 0.90$ in a population of Ng = 75, in close accordance with predictions (D. Garant et al., unpublished data). Estoup et al. (1998) observed that approximately four and five loci were necessary to reach $P = 0.90$, in situations where the number of parents was approximately 30, and mean allelic diversity was 9 and 14, respectively, for two different species. This is also in close accordance with the predictions of our model.

Clearly, however, this model may be improved to take into account other parameters. Namely, the model is based on simple assumptions of the Wright–Fisher random mating model. Note, however, that this mating pattern represents the most stringent situation for allocation success, since the number of possible matings for a given number of parents would be reduced for any other type of mating scheme. A first and obvious extension would be to consider sexed parents, unequal sex ratio, and various reproductive patterns, such as factorial and paternity retrieval scheme (Sancristobal and Chevalet 1997; Estoup et al. 1998). The model could also be modified specifically for paternity studies in which maternal–offspring relationships are known or not (Marshall et al. 1998). In microsatellite studies, allelic scoring errors are not rare (O'Reilly et al. 1998) and may significantly decrease the proportion of allocation success (Sancristobal and Chevalet 1997; Marshall et al. 1998). At this time, we cannot predict the effect of the rate of scoring error on Ps, given specific N, n, and Ng values. Such knowledge would be of great use for predictive as well as theoretical purposes. To minimize the negative impact of scoring errors, this investi-

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gation should be led within the framework of an error tolerant allocation procedure (Sancristobal and Chevalet 1997), which excludes few, if any, potential pairs, such that a partly mistaken offspring genotype may still be correctly allocated. Finally, the proposed model assumes that all possible parents are identified and genotyped. Clearly, this assumption will not always be satisfied in wild populations. It will certainly be of practical concern to be able to predict the loss of success rate based on at least approximate estimates of the proportion of missing potential parents (Marshall et al. 1998).

For population assignment, the expectancy of the proportion of population allocation success has been modelled as $P(N, n)$, a function of number of loci and average number of alleles per locus in the specific case of two populations. We observed a relatively high concordance between predicted and observed proportion of allocation success. Thus, the differences between predicted and observed absolute values did not exceed 0.03 over the range of number of loci and alleles covered in the study. One important feature of the model is that it predicts an additive contribution of the number of loci and number of alleles per locus. For a fixed number of loci, the contribution of number of alleles to the total proportion of allocation success never exceeds 6%, and this value is reached soon, approximately at $n = 6$. Clearly, the contribution to allocation success of the number of alleles is largely outweighed by that of the number of loci N. The structure of relationships between N, n, and Ps is thus very different from the one prevailing in the case of parentage assignment where we observed that the number of alleles could always be made large enough to get $P \approx 1$ and a strong interactivity between N and n. In brief, while the use of loci with high allelic diversity is highly advantageous in studies of parentage assignment, there is no apparent gain of doing so beyond a given level (approximately $n = 6$) in population assignment studies. This prediction only stands in a situation of two populations. Although not explored here, our prediction is that maximum gain will be obtained with values of $n > 6$ in situations with more than two populations. However, the asymptotic relationships between P and n would most likely remain.

While the contribution of number of loci to allocation success largely outweighs that of the number of alleles, we observed that the variance in Ps quickly decreased with an increase in both n and N, these two variables being practically interchangeable in this respect. This means that the predictive precision of the model grows with allocation success itself. Consequently, more than 80% of Ps values fell within the interval $(P - 0.05, P + 0.05)$ when $P \geq 0.85$. Thus, an increase in average number of alleles, while adding little to E(Ps), reduces the uncertainty in predicting allocation success. On the other hand, this positive asset may potentially be counterbalanced by sampling errors of low frequency alleles that could reduce allocation success when using maximum likelihood methods (Smouse and Chevillon 1998; Roques et al. 1999). Consequently, we conclude that the best strategy for optimizing allocation success in studies of population assignment is to use loci with moderate allelic diversity, with n varying between 6 and 10 as a rule of thumb.

As for parentage assignment, the model that we developed to predict allocation success in studies of population assignment should be improved to take into account other parameters. In practice, such studies will often involve more than two populations, and consequently, our model could be extended to any reasonable number of populations. A preliminary exploration by simulation approach indicated that the general structure of relationships between Ps, N, and n is not altered with a varying number k of populations. This means that the generalization of $P(N, n)$ to a suitable $P(N, n, k)$ should be possible. Also, we did not attempt to predict allocation success as a function of various levels of population divergence. Clearly, allocation success is expected to increase as a function of population divergence (Paetkau et al. 1995; Smouse and Chevillon 1998; Roques et al. 1999).

To conclude, there is no doubt that in the years to come, the use of individual multilocus information will increasingly contribute importantly to our knowledge of fundamental issues of the biology of aquatic organisms. Such information will in turn contribute to improving management, conservation, and production practices. The use of predictive tools, such as the first generation of models developed here, should contribute to maximizing the returns of such applications by increasing our understanding of interactions among the various variables affecting allocation success and by allowing the adjustment a priori of the required levels of resolution and, consequently, optimizing the costs–benefits ratio in the use of molecular markers.

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References

Amos, B., Schlötterer, C., and Tautz, D. 1993. Social structure of pilot whales revealed by analytical DNA profiling. Science (Washington, D.C.), **260**: 670–672.

Banks, M.A., Baldwin, B.A., and Hedgecock, D. 1996. Research on chinook salmon (*Oncorhynchus tshawytscha*) stock structure using microsatellite data. Bull. Natl. Res. Inst. Aquacult. **2**(Suppl.): 5–9.

Bernatchez, L. 1995. A role for molecular systematics in defining evolutionarily significant units in fishes. *In* Evolution and aquatic ecosystem: defining units in population conservation. *Edited by* J.L. Nielsen. Am. Fish. Soc. Symp. **17**: 114–132.

Bernatchez, L., and Wilson, C.C. 1998. Comparative phylogeography of nearctic and palearctic fishes. Mol. Ecol. **7**: 465–452.

Bernatchez, L., Chouinard, A., and Lu, L. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. Biol. J. Linn. Soc. **68**: 173–197.

Bowen, B.W., and Avise, J.C. 1995. Conservation genetics of marine turtles. *In* Conservation genetics: case histories from nature. *Edited by* J.C. Avise and J.L. Hamrick. Chapman and Hall, New York. pp. 190–237.

Carvalho, G.R., and Hauser, L. 1994. Molecular genetics and the stock concept in fisheries. Rev. Fish Biol. Fish. **4**: 326–350.

Clapham, P.J., and Palsbøll, P.J. 1997. Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). Proc. R. Soc. Lond. Ser. B, Biol. Sci. **264**: 95–98.

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Colbourne, J.K., and Hebert, P.D.N. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Philos. Trans. R. Soc. Lond. B, Biol. Sci. **351**: 349–360.

Davies, N., Villablanca, F.X., and Roderick, G.K. 1999. Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. Trends Ecol. Evol. **14**: 17–21.

Dizon, A.E., Lockyer, C., Perrin, W.F., Demaster, D.P., and Sisson, J. 1992. Rethinking the stock concept: a phylogeographic approach. Conserv. Biol. **6**: 24–36.

Estoup, A., and Angers, B. 1998. Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. *In* Advances in molecular ecology. *Edited by* G.R. Carvalho. NATO Sciences Series, IOS Press, Amsterdam, The Netherlands. pp. 55–79.

Estoup, A., Gharbi, K., SanCristobal, M., Chevalet, C., Haffrey, P., and Guyomard, R. 1998. Parentage assignment using microsatellites in turbot (*Scophtalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) hatchery populations. Can. J. Fish. Aquat. Sci. **55**: 715–725.

Ferguson, M.M., and Danzmann, R.G. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Can. J. Fish. Aquat. Sci. **55**: 1553–1563.

Foltz, D.W., and Hoogland, D.W. 1981. Analysis of the mating system in the black-tailed prairie dog (*Cynomis ludovicianus*) by likelihood of paternity. J. Mammal. **62**: 706–712.

Fontaine, P.M., and Dodson, J.J. 1999. An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites. Mol. Ecol. **8**: 189–198.

Hanken, J., and Sherman, P.W. 1981. Multiple paternity in Belding's ground squirrel litters. Science (Washington, D.C.), **212**: 351–353.

Herbinger, C.M., Doyle, R.W., Taggart, C.T., Lochmann, S.E., Wright, J.M., Brooker, A.L., and Cook, D. 1997. Family relationships and effective population size in a natural cohort of cod larvae. Can. J. Fish. Aquat. Sci. **54**: 11–18.

Jones, A.G., and Avise, J.C. 1997. Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role inversal. Mol. Ecol. **6**: 203–213.

Jones, A.G., Kvarnemo, C., Moore, G.I., Simmons, L.W., and Avise, J.C. 1998. Microsatellite evidence for monogamy and sex-biased recombinaition in the Western Australian seahorse *Hippocampus angustus*. Mol. Ecol. **7**: 1497–1505.

Jordan, W.C., and Youngson, A.F. 1992. The use of genetic marking to assess the reproductive success of mature male Atlantic salmon parr (*Salmo salar* L.) under natural spawning conditions. J. Fish Biol. **41**: 613–618.

Lu, G., and Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution, **53**: 1491–1505.

Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. **7**: 639–655.

Mayden, R.L. 1995. *In* Evolution and aquatic ecosystem: defining units in population conservation. *Edited by* J.L. Nielsen. Am. Fish. Soc. Symp. **17**: 114–132.

Meagher, T.R., and Thompson, E. 1986. The relationship between single parent and parent pair genetic likelihoods in genealogy reconstruction. Theor. Popul. Biol. **29**: 87–106.

Nielsen, E.E., Hansen, M.M., and Loeschcke, V. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years. Mol. Ecol. **6**: 487–492.

O'Reilly, P.T., Herbinger, C., and Wright, J.M. 1998. Analysis of parentage determination in Atlantic salmon (*Salmo salar*) using microsatellites. Anim. Genet. **29**: 363- 370.

Paetkau, D., Calvert, W., Stirling, I., and Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. **4**: 347–354.

Palsbøll, P.J., Allen, J., Bérubé, M., Clapham, P.J., Feddersen, T.P., Hammond, P.S., Hudson, R.R., Jørgensen, H., Katona, S., Larsen, A.H., Larsen, F., Lien, J., Mattila, D.K., Sigurjònsson, J., Sears, R., Smith, T., Sponer, R., Stevick, P., and Øien, N. 1997. Genetic tagging of humpback whales. Nature (Lond.), **388**: 767–769.

Rico, C., Kuhnlein, U., and Fitzgerald, G.J. 1992. Male reproductive tactics in the threespine stickleback — an evaluation by DNA fingerprinting. Mol. Ecol. **1**: 79–87.

Roques, S., Duchesne, P., and Bernatchez, L. 1999. Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study. Mol. Ecol. **8**: 1703–1718.

Sancristobal, M., and Chevalet, C. 1997. Error tolerant parent identification from a finite set of individuals. Genet. Res. **70**: 53–62.

Shriver, M.D., Smith, M.W., Jin, L., Marcini, A., Akey, J.M., Deka, R., and Ferrell, R.E. 1997. Ethnic-affiliation estimation by use of population-specific DNA markers. Am. J. Hum. Genet. **60**: 957–964.

Smouse, P.E., and Chakraborty, R. 1986. The use of restriction fragmment length polymorphisms in paternity analysis. Am. J. Hum. Genet. **38**: 918–939.

Smouse, P.E., and Chevillon, C. 1998. Analytical aspects of population-specific DNA fingerprinting for individuals. J. Hered. **89**: 143–150.

Smouse, P.E., Spielman, R.S., and Park, M.-H. 1982. Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. Am. Nat. **119**: 445–463.

Stanley, H.F., Casey, S., Carnahan, J.M., Goodman, S., Harwood, J., and Wayne, R.K. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). Mol. Biol. Evol. **13**: 368–382.

Taylor, E.B., and Bentzen, P. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution, **47**: 813–832.

Taylor, E.B., and McPhail, J.D. 1999. Evolutionary history of an adaptive radiation in sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA variation. Biol. J. Linn. Soc. **66**: 271–291.

Tessier, N., and Bernatchez, L. 1998. Contribution des différentes populations de ouananiches à la pêche sportive en lac pour les années 1994 à 1996. Rapport présenté par l'Université Laval à la Corporation de l'Activité Pêche Lac Saint-Jean (CLAP). Université Laval, Sainte-Foy, Qué.

Turgeon, J., Estoup, A., and Bernatchez, L. 1999. Species flock in the North American Great Lakes: molecular ecology of lake Nipigon ciscoes (Teleotei: Coregonidae: *Coregonus*). Evolution, **53**: 1857–1871.

Utter, F., and Ryman, N. 1993. Genetic markers and mixed fisheries. Fisheries (Bethesda), **18**: 11–21.

Ward, R.D., and Grewe, P.M. 1994. Appraisal of molecular genetic techniques in fisheries. Rev. Fish Biol. Fish. **4**: 300–325.

Waser, P.M., and Strobeck, C. 1998. Genetic signatures of interpopulation dispersal. Trends Ecol. Evol. **13**: 43–44.

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Appendix 1. Minimum number of loci to reach at least 90% of successful allocations as $N_{norm}(n, Ng, 0.83)$ rounded to the nearest integer

Number of alleles	Number of parents					
	50	100	150	200	250	300
2	32	37	40	41	42	43
3	19	22	23	24	25	25
4	13	16	17	18	18	18
5	11	12	13	14	14	14
6	9	10	11	12	12	12
7	8	9	10	10	10	11
8	7	8	9	9	9	9
9	6	7	8	8	8	9
10	6	7	7	8	8	8
11	5	6	7	7	7	7
12	5	6	6	7	7	7
13	5	6	6	6	6	7
14	5	5	6	6	6	6
15	4	5	6	6	6	6
16	4	5	5	6	6	6
17	4	5	5	5	5	6
18	4	5	5	5	5	5
19	4	4	5	5	5	5
20	4	4	5	5	5	5

Appendix 2. Maximum likelihood parental allocation procedure

Compute the likelihood of an offspring genotype for each pair of parental genotypes
Given the offspring genotype

$$Go = ([R1, R2], [R3, R4], [R5, R6],...)$$

and the two parental genotypes

$$Par1 = ([M1, M2], [M3, M4], [M5, M6],...)$$

$$Par2 = ([F1, F2], [F3, F4], [F5, F6],...)$$

we first compute the following for locus 1:

$$Pr = (1/2pr(M1 \rightarrow R1) + 1/2pr(M2 \rightarrow R1)) \times (1/2pr(F1 \rightarrow R2) + 1/2pr(F2 \rightarrow R2)) + (1/2pr(F1 \rightarrow R1) + 1/2pr(F2 \rightarrow R1)) \times (1/2pr(M1 \rightarrow R2) + 1/2pr(M2 \rightarrow R2))$$

where

$pr(A \rightarrow B) = 1$ if $A = B$, $= 0$ if $A \neq B$, assuming mendelian transmission.

The likelihood $L1$ of Go genotype at the first locus among the possible progeny of the parental pair (Par1, Par2) is

$L1 = Pr$ if locus 1 is a heterozygote for Go

$L1 = Pr/2$ if locus 1 is a homozygote for Go.

The global likelihood of Go, given the parental pair (Par1, Par2), is the product of all single locus likelihoods:

$$L(Go) = L1 \times L2 \times L3 \times... \times Ln.$$

The above computation has to be done for each potential parental pair.

Allocation of offspring Go

Once the set of all likelihoods (one to each parental pair) has been calculated, the highest one is retained. If this maximum belongs to a single parental pair, the offspring is allocated to the latter; otherwise, it is not allocated. Lack of allocation is equivalent to an incorrect allocation when estimating Ps by running Monte-Carlo simulations.

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Land use, fishing, climate change, and the decline of Thompson River, British Columbia, coho salmon

Michael J. Bradford and James R. Irvine

Abstract: We investigated a recent, major decline in the abundance of a large aggregate of coho salmon (*Oncorhynchus kisutch*) spawning in the Thompson River, British Columbia, watershed. We found that the decline could be attributed to a declining trend in productivity likely related to changing ocean conditions, overfishing, and freshwater habitat alteration. Among individual watersheds, rates of decline in adult coho salmon abundance were correlated with agricultural land use, road density, and a qualitative measure of stream habitat status but not with the proportion of land recently logged. The recovery of these populations will require the prudent regulation of fishing, the restoration of salmon producing watersheds, and an improvement in ocean conditions.

Résumé : Nous avons examiné le déclin marqué de l'abondance qui vient de se manifester chez un important groupe de cohos (*Oncorhynchus kisutch*) qui frayent dans le bassin de la Thompson (Colombie-Britannique). Nous pensons que ce déclin pourrait s'expliquer par une tendance à la baisse de la productivité, tendance qui semble reliée aux changements dans les conditions océaniques, à la surpêche et à l'altération des habitats d'eau douce. Au niveau des bassins, les taux de déclin de l'abondance des cohos adultes étaient corrélés à l'utilisation des terres agricoles, à la densité du réseau routier et à une mesure qualitative de l'état des habitats d'eau douce, mais pas à la proportion de territoire forestier récemment mis en coupe. Le rétablissement de ces populations nécessitera une réglementation prudente de la pêche, une remise en état des bassins salmonicoles et une amélioration des conditions océaniques.

[Traduit par la Rédaction]

Introduction

Many Pacific salmon (*Oncorhynchus* spp.) populations have declined to critical levels. The cause of the declines are usually thought to be some combination of overfishing, decreased survival of young salmon in the ocean, and degraded freshwater habitat (Slaney et al. 1996), though other hypotheses have been proposed (Walters and Ward 1998).

The abundance of coho salmon (*O. kisutch*) returning to, and spawning in, the Thompson River watershed has declined 90% in the last decade (Fig. 1*a*). This decline has recently forced major restrictions on Canadian salmon fisheries that can catch these fish. While it is often difficult to determine the relative roles of the various factors on trends in adult salmon abundance, here, we provide quantitative evidence that fishing, climate change, and habitat alterations have all contributed to the decline. In particular, we document quantitative relations between large-scale land use patterns and trends in adult salmon abundance.

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Methods

The Thompson River is the largest tributary of the Fraser River and drains 54 600 km^2 of the mountainous southern interior of British Columbia. Coho salmon spawn in the fall in at least 40 streams and rivers in the Thompson drainage, and most juveniles spend a year in freshwater before migrating to the sea as smolts. Subadults rear in the North Pacific and most return back to the Thompson River at a total age of 3 years.

Much of our analysis is based on estimates of abundance derived from visual counts of spawners. There is considerable uncertainty in these data, and most estimates are probably biased low. However, in many cases, observers worked in the same locations for many years, and Irvine et al. (1999*a*) detailed the efforts that have been made to make the data consistent as possible over time. We created four time series of spawner abundances: the sum of estimates from 10 North Thompson streams, the sum of 19 South Thompson streams, the Eagle River, and the Salmon River. Data were available for these streams for at least 11 of 14 years from 1985–1998 (Irvine et al. 1999*a*, 1999*b*); missing data were linearly interpolated from the adjacent years. Spawner estimates for the Eagle and the Salmon rivers were based on weirs and are likely more accurate than for the other streams. These two streams also had hatchery operations on them; however, return rates of hatchery fish were low and had little effect on overall adult abundances or trends (Bradford 1998). The other streams were free of hatchery interventions.

We calculated recruitment (R) by expanding spawner estimates by the exploitation rate as $R_t = S_t/(1-h_t)$, where R_t is the abundance of maturing adults returning to coastal waters, S_t is spawner abundance, and h_t is the proportion of fish taken in all fisheries that year. Exploitation rate estimates are from coded-wire tag recoveries from Thompson hatchery smolt releases (Irvine et al. 1999*b*) supplemented by DNA sampling of catches in fisheries during 1998 (Irvine et al. 1999*a*). We evaluated the possible impacts of climate change by calculating the intrinsic rate of increase, r_t =

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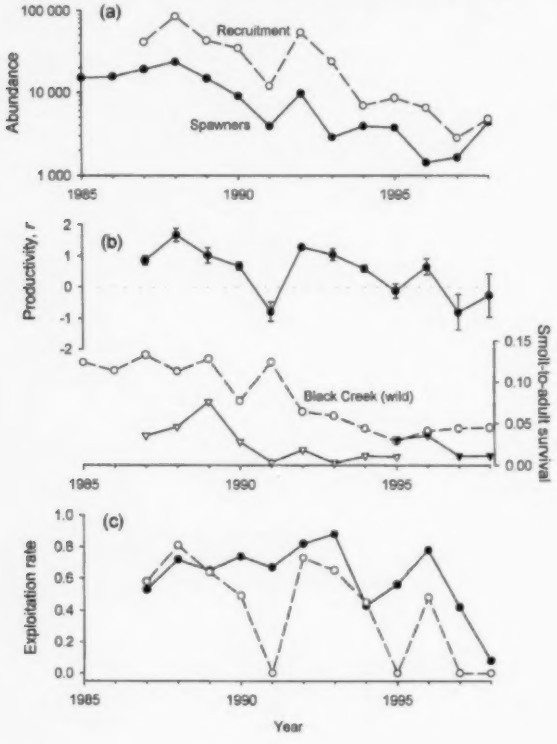
Fig. 1. (*a*) Total abundance of coho salmon recruits and spawners for 31 Thompson streams. (*b*) Productivity r (±SE) averaged over four population aggregates (North Thompson, South Thompson, Eagle, and Salmon). Also shown are smolt-to-adult survival rates for a Strait of Georgia wild coho population (broken line) and for North (▼) and South (▽) Thompson hatchery releases (solid lines). (*c*) Fishery exploitation rates for Thompson hatchery releases (●); open circles are the annual exploitation rates that would have maintained wild spawner abundances (h^*).



$\ln(R_t/S_{t-3})$, which is an annual measure of productivity. We computed r for each of the four time series of abundance and then averaged them.

The appropriateness of harvest rates is usually determined with a stock–recruit model that predicts the response of populations to exploitation under average or stationary conditions. However, there was a strong trend in the productivity of Thompson coho salmon (Fig. 1*b*), which precluded such an analysis. Instead, we used the annual estimates of r to calculate the harvest rate, $h^* = 1 - e^{-r}$ ($h^* = 0$ if $r \leq 0$). For years when $r > 0$, h^* would have maintained the populations at stable levels (i.e., $S_t = S_{t-3}$). We then compared h^* with the actual exploitation rates. We considered fishing to have contributed to the decline in abundance when the observed values of h exceeded h^*.

We evaluated the role of land use on coho salmon declines by comparing different measures of land use in each watershed with the rate of change in the abundance of adults returning to that stream. We hypothesized that variation in the rates of decline in recruitment among streams would be related to the quality of freshwater habitat, which would affect egg-to-smolt survival. Because the exact mechanisms by which land use impacts aquatic habitats are diverse and probably watershed specific, we used only the most general land use measures for this analysis.

For each stream we calculated the rate of change of estimated recruitment as the slope of the regression of $\ln(R+1)$ on year for the years 1988–1998. We had data for 40 streams for which estimates were available for at least 7 of the 11 years. We used 3 measures of human disturbance for each watershed that were obtained from a GIS database (www.gdbc.gov.bc.ca): (*i*) the proportion of land in agricultural and urban use, (*ii*) the density of roads, and (*iii*) the proportion of land that had been logged or burned in the last 20–25 years. The logging, agricultural, and urban data were based on remote sensing information collected in 1994–1995. Road data (including hard surface, logging, and farm roads) were from aerial photography conducted in 1988 and will underestimate road lengths in watersheds with recent logging. For larger watersheds, the proportion of land in agricultural use was estimated for the mainstem area only and does not include small higher gradient tributary streams that are not extensively used by coho or for farming. Logging and road densities were estimated for the whole watershed because of the potential for upland land use to impact downstream areas.

We used a semiquantitative index of "habitat concerns" for each watershed as a fourth measure. This independent index comprised 10 major categories (forestry, agriculture, urbanization, recreation, mining, industrial development, linear development, hydro development, cumulative impacts, and special biophysical concerns), and there were one to six subcategories in each major group (Department of Fisheries and Oceans 1998*a*, 1998*b*). Each issue was rated as either "high" or "low," and the watershed score was the sum of the number of "high" concerns. The watershed index was not available for part of the Thompson drainage area, so we asked a biologist who was not familiar with the fish data to review the supporting documents and generate the index values in a manner similar to what was done for the other areas.

Six of the 40 streams were sites for hatchery operations of varying intensities. To test for an effect of the hatchery on the rate of decline in abundance, we used multiple regressions with one of the land use measures and a dummy variable coded for hatchery presence as independent variables.

Results

The productivity of wild Thompson coho has declined to the point that now $r \approx 0$, which means populations are only just able to replace themselves in the absence of any fishing mortality (Fig. 1*b*). When we compared the actual exploitation rates with our estimates of h^*, we found that fishing mortality was well matched to the productivity of the aggregate between 1987 to 1989, but since then, harvest rates have often been excessive. The median difference between the observed exploitation rates and h^* (1990–1998) was 0.25.

The rate at which individual coho populations declined between 1988 and 1998 was related to the extent of agricultural and urban land use, the density of roads in the watershed, and the index of habitat concerns, but there was no relationship between salmon declines and recent logging (Fig. 2). The presence of hatchery activities had no effect on the rates of decline (for all four measures of land use, the dummy variable for hatchery presence was not significant, $P > 0.15$). Decline rates were not related to watershed area ($r = -0.01$). The agricultural, road, and habitat concern measures were correlated with each other ($r = 0.53$–0.74). The index of recent logging was weakly correlated with road density ($r = 0.35$) but was not with the agriculture or habitat concern indices ($r = -0.14$ and -0.06, respectively).

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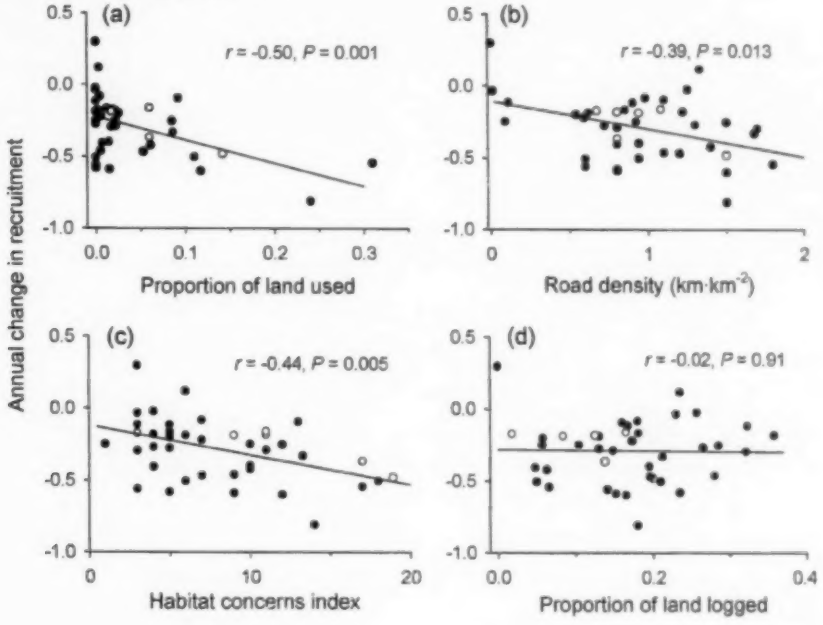
Fig. 2. Relationship between the instantaneous average annual rates of change of coho salmon recruitment (1988–1998) and four indices of land use for 40 Thompson watersheds. Lines are the least-squares linear regression equations. (*a*) Proportion of land under agricultural and urban use, (*b*) density of roads, (*c*) habitat concerns index, (*d*) proportion of land recently logged or burned. Open circles are streams that were subject to hatchery programs.



Discussion

The productivity of the Thompson coho aggregate has declined steadily in the past decade. The decline in *r* is not due to density-dependent mortality in freshwater because *r* and population sizes show concurrent downward trends. We also do not believe that the trend in *r* could be due to a recent basin-wide deterioration in physical habitat quality. Rather, we think the trend in *r* is due to changes in climate, particularly as it affects ocean conditions. We do not have direct estimates of the survival of wild coho once they leave the Thompson drainage, but smolt-to-adult survival rates for Thompson hatchery releases have declined, as has survival for a wild population from the Georgia Strait, British Columbia (Fig. 1*b*). The survival of other coho salmon populations from southern British Columbia has also decreased significantly in recent years (Walters and Ward 1998). There is also evidence of very recent (since 1995) declines in body size, fecundity, and the proportion of females in Thompson coho salmon spawning populations (Irvine et al. 1999*b*). These changes are likely due to changes in ocean conditions and will affect the productivity of the aggregate in the coming years. Climate change might also affect the survival of coho salmon in freshwater (Walters and Ward 1998), but quantitative evidence for large-scale climate effects on the production of coho salmon smolts is lacking (Bradford 1999).

Among individual watersheds, coho salmon declines were related to the intensity of human disturbance in the catchment. Juvenile coho salmon favour low gradient streams that are located on valley floors in mountainous regions (Beechie et al. 1994). These areas are also preferred for agriculture, forestry, urban development, and transportation corridors; all are activities that can impact aquatic habitats. In the Thompson watershed, many valley bottoms have been in use for agriculture (mainly livestock, dairy, and animal feed crops) for at least 50 years. In some cases, riparian vegetation has been removed and livestock has destabilized stream banks, and off-channel habitats and wetlands have been destroyed or isolated by dyking. In addition, much of the Thompson drainage is in a semiarid area, and high rates of water withdrawal in summer for irrigation cause low flows and high water temperatures (Rood and Hamilton 1995).

Although we could not detect an effect, we do not suggest that logging in the Thompson watershed has not had an impact on coho streams. The index that we used concerns only recent logging, which was often located in the headwaters of salmon streams, as the forests that covered most valley floors were removed 50–100 years ago for agricultural and rangeland uses. The effects of recent logging in headwaters may be too subtle to detect with our data, as the impacts can be varied and can occur over long time scales (Meehan 1992). Older deforestation has been shown to affect aquatic biota (Harding et al. 1998), but data to quantify 50- to 100-year-old logging were not available to us. Further analyses, and perhaps a better understanding of the impacts of logging in interior climates, are needed (Meehan 1992) to fully evaluate the effects of logging in the Thompson basin.

A provisional estimate of the contribution of landscape alteration to the rate of change in coho salmon abundance can be made by comparing the observed instantaneous rate of change averaged across all streams (–0.29) with the inter-

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cepts of the regressions of Figs. 2*a*–2*c*, which predict the average rate of decline in pristine watersheds. These intercepts range from –0.11 to –0.22, which implies that Thompson coho salmon populations would have still declined, although at a lesser rate, if stream habitats had been pristine.

We assumed that fish spawning in each stream represent individual populations, but it is likely that there is exchange of juveniles and adults among neighboring streams within the drainage. Thus the Thompson coho salmon aggregate may actually consist of a smaller number of larger populations in which "source-sink" processes may be important for local population dynamics. Freshwater habitat deterioration could alter the source-sink balance and may reduce connectivity among populations, which will affect the overall productivity and stability of the aggregate. Unfortunately, we know little about these processes for Thompson River coho salmon.

In summary, we find evidence that excessive fishing, changing ocean conditions, and freshwater habitat alterations have all contributed to declines in Thompson River coho salmon. Overfishing resulted from the failure of harvest rates to track a climate-driven decline in productivity. The status of freshwater habitats has contributed to the resilience of individual populations, and our results highlight the critical role that productive freshwater habitats can play in aiding salmon populations during periods of unfavourable ocean conditions. Unfortunately, because we lack a population model to link generations, we cannot more quantitatively apportion the overall decline to each of the three factors. Nonetheless, it seems prudent to suggest that the recovery of these populations will require continued restrictions on fishing, the restoration of salmon producing watersheds, and an improvement in ocean conditions.

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References

Beechie, T.E., Beamer, E., and Wasserman, L. 1994. Estimating coho salmon rearing habitat and smolt production losses in a large river basin, and implications for habitat restoration. N. Am. J. Fish. Manage. **14**: 797–811.

Bradford, M.J. 1998. Risk analysis for Thompson coho salmon. Can. Stock Assess. Secretariat (CSAS) Res. Doc. 98/92. (Available from CSAS, 200 Kent St., Ottawa, ON K1A 0E6.)

Bradford, M.J. 1999. Temporal and spatial trends in the abundance of coho salmon smolts from western North America. Trans. Am. Fish. Soc. **128**: 840–846.

Department of Fisheries and Oceans (DFO). 1998*a*. Salmon watershed planning profiles for the South Thompson–Shuswap Habitat Management Area. (Available from Fraser River Action Plan, DFO, 555 West Hastings St., Vancouver, BC V6B 5G3, Canada.)

Department of Fisheries and Oceans (DFO). 1998*b*. Salmon watershed planning profiles for the Thompson Nicola Habitat Management Area. (Available from Fraser River Action Plan, DFO, 555 West Hastings St., Vancouver, BC V6B 5G3, Canada.)

Harding, J.S., Benfield, E.F., Bolstad, P.V., Helfman, G.S., and Jones, E.B.D. 1998. Stream biodiversity: the ghost of land use past. Proc. Natl. Acad. Sci. U.S.A. **95**: 14 843 – 14 847.

Irvine, J.R., Bailey, R.E., Bradford, M.J., Kadowaki, R.K., and Shaw, W.S. 1999*a*. 1999 Assessment of Thompson River/Upper Fraser River Coho Salmon. Can. Stock Assess. Secretariat Res. Doc. 99/128. (Available from CSAS, 200 Kent St., Ottawa, ON K1A 0E6, Canada.)

Irvine, J.R., Wilson, K., Rosenberger, B., and Cook, R. 1999*b*. Stock assessment of Thompson River/Upper Fraser River coho salmon. Can. Stock Assess. Secretariat Res. Doc. 99/28. (Available from CSAS, 200 Kent St., Ottawa, ON K1A 0E6, Canada, or www.dfo-mpo.gc.ca/csas.)

Meehan, W.R. 1992. Influences of forest and rangeland management on salmonid fishes and their habitats. Am. Fish. Soc. Spec. Publ. 19.

Rood, K.M., and Hamilton, R.E. 1995. Hydrological and water use for salmon streams in the Thompson River watershed, British Columbia. Can. Man. Rept. Fish. Aquat. Sci. 2297.

Slaney, T.L., Hyatt, K.D., Northcote, T.G., and Fielden, R.J. 1996. Status of anadromous salmon and trout in British Columbia and Yukon. Fisheries, **21**(10): 20–35.

Walters, C., and Ward, B. 1998. Is solar radiation responsible for declines in marine survival rates of anadromous salmonids that rear in small streams? Can. J. Fish. Aquat. Sci. **55**: 2533–2538.

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Can reduced salmonid population abundance be detected in time to limit management impacts?

Kenneth D. Ham and Todd N. Pearsons

Abstract: We evaluated eight populations of native salmonids to determine if rapid, sensitive detection of a reduction in abundance is possible in the Yakima River basin, Washington, where a large-scale test of hatchery supplementation is being conducted. Prospective power to detect impacts to abundance was estimated from 3–16 annual baseline surveys conducted by electrofishing, trapping, or snorkeling. High interannual variation in abundance estimates (CV = 26–94%) prevented detection of small impacts for most taxa. For three taxa, models of environmental and biological influences accounted for between 42 and 49% of temporal variation, increasing our ability to detect impacts of other influences. Detectable impacts for a *t* test with $\alpha = 0.1$ and $\beta = 0.1$ were >18% for all eight taxa and >54% for four of eight taxa. We suggest that population abundance monitoring may not provide feedback sufficiently sensitive or rapid enough to implement corrective actions that prevent impacts from causing harm or exceeding an acceptable level, especially for rare or highly valued taxa with small acceptable impacts.

Résumé : Nous avons évalué huit populations de salmonidés indigènes dans le but de déterminer s'il était possible de procéder à une détection rapide et sensible de toute réduction de l'abondance dans le bassin de la rivière Yakima (Washington) où l'on procède à un essai à grande échelle d'apports de poissons d'élevage. La capacité de déceler des effets sur l'abondance a été estimée à partir de 3 à 16 relevés de base annuels effectués par le moyen de la pêche électrique, du piégeage et de l'observation par plongeur. L'importante variation interannuelle des estimations d'abondance (CV = 26 à 94%) a empêché de déceler les petits effets sur la plupart des taxons. Pour trois taxons, des modèles des effets environnementaux et biologiques permettaient de rendre compte de 42 à 49% de la variation temporelle, ce qui augmentait notre capacité à déceler les effets d'autres influences. Les effets décelables, pour un test *t*, où $\alpha = 0,1$ et $\beta = 0,1$, étaient >18% pour les huit taxons et >54% pour quatre des huit taxons. Nous formulons l'hypothèse que le contrôle de l'abondance de la population pourrait ne pas donner des résultats suffisamment sensibles ou rapides pour permettre d'apporter des mesures correctives visant à prévenir des effets nuisibles ou supérieurs à un niveau acceptable, tout particulièrement pour des taxons rares ou de grande valeur lorsque les effets acceptables sont faibles.

[Traduit par la Rédaction]

Introduction

Stocking of hatchery-reared anadromous salmonids to supplement wild populations has been identified as a major strategy to restore, maintain, and conserve imperiled anadromous salmonids in the Pacific Northwest (Regional Assessment of Supplementation Planning 1992; Northwest Power Planning Council 1994; Fast and Craig 1997). The use of artificial propagation in an attempt to maintain or increase natural production is termed "supplementation" (Regional Assessment of Supplementation Planning 1992). Supplementation is untested and is not without controversy (Independent Scientific Group 1996; National Research Council 1996; Busack et al. 1997). Part of the controversy reflects the concern of how cultured fish (target taxa) may impact wild fishes that are not being supplemented (nontarget taxa) (White et al. 1995; McMichael et al. 1997). Supplementation has frequently been coupled with adaptive management because of the high scientific uncertainty of outcomes (Regional Assessment of Supplementation Planning 1992; Bonneville Power Administration 1996). Adaptive management is founded upon the ability to design management actions as experiments and learn from mistakes so that corrections can be made (Walters and Hilborn 1976; Walters 1986). Rapid, sensitive detection of reductions in abundance is needed if we wish to implement corrective action in time to prevent these impacts from progressing to a level that harms the population of interest or exceeds an acceptable level.

As with other ecological experiments, monitoring to detect reduced abundance faces difficulty in establishing sufficient replication and controls (Hurlbert 1984), sampling the component of interest (Eberhardt and Thomas 1991), and overcoming high levels of variation unrelated to the imposed treatment. Frequently, environmental monitoring plans are designed with insufficient statistical power (Peterman and Bradford 1987; Peterman 1990). Insufficient power can lead to a failure to reject a false null hypothesis (i.e., an impact goes undetected), a type II statistical error (Peterman 1990). If this error occurs, impacts are underestimated, possibly masking the need for corrective action. Controlling or recognizing the probability of a type II error is essential to evaluating the potential risk of a management action, especially

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where conservation of a species is involved (National Research Council 1995). Therefore, the need exists to evaluate, for each monitoring design, whether impact detection will be rapid and sensitive enough to allow corrective actions to be implemented.

Impact detection is affected by temporal variation in population status in the absence of the impact of interest, identification of sources of variation that explain nonimpact variation, and baseline data quantity and quality. Experimental designs are weak when no experimental controls are available, as is frequently the case when working in large river systems, and impacts must be determined primarily from data collected before and after a management action (Green 1979; Eberhardt and Thomas 1991; Stewart-Oaten et al. 1992; Stewart-Oaten 1996). This is the case in the Yakima River basin where supplementation of spring chinook salmon (*Oncorhynchus tshawytscha*) has been in the planning stages for over a decade (Clune and Dauble 1991; Fast and Craig 1997).

The Yakima River basin is an appropriate place to evaluate the limits of population abundance monitoring in detecting impacts. First, a tremendous amount of data on a variety of species has been collected (Fast et al. 1991; Bonneville Power Administration 1996; Busack et al. 1997; Pearsons et al. 1998). Second, the Yakima Fisheries Project was designed to produce information and methodologies that could be used in other areas of the Columbia River basin (Busack et al. 1997). Third, the baseline sampling period has ended, and detectable impacts can now be estimated. In this paper, we evaluate whether monitoring the abundance of native salmonid populations is likely to detect an impact before it exceeds an acceptable level. The supplementation project in the Yakima River will be used as a case study to illustrate the potential of using abundance monitoring for detecting ecological impacts.

Methods

Study area and background

The Yakima River is the longest river located entirely within Washington and flows in a north to south direction before entering the Columbia River near the city of Richland (Fig. 1). The Yakima River drainage basin has an area of 15 900 km^2 and ranges from over 2440 m in elevation at the headwaters to about 104 m at the river mouth. Forty-eight fish species have been identified in the Yakima basin, of which only 29 are of native origin (Pearsons 1998). Among these species, salmonids are the most studied and are some of the most important to humans. This study includes eight populations of native salmonids, of which three are anadromous and five are resident.

As in other parts of the Pacific Northwest, many salmon populations in the Yakima River basin have declined to a fraction of their historical abundance. For instance, bull trout (*Salvelinus confluentus*) is federally listed as threatened, and the mid-Columbia steelhead (*Oncorhynchus mykiss*) has recently been listed as threatened under the Endangered Species Act (National Marine Fisheries Service 1999; U.S. Fish and Wildlife Service 1998). Westslope cutthroat trout (*Oncorhynchus clarki*) has been petitioned for listing. Species that are still abundant support important fisheries. For example, rainbow trout (*O. mykiss*) in the mainstem Yakima River provides one of the best resident wild trout fisheries in the state of Washington (Krause 1991; Probasco 1994) and is currently managed as a catch and release fishery. Chinook salmon is also taken in Yakima basin subsistence fisheries and is utilized in recreational and commercial fisheries in the Columbia River and Pacific Ocean (Hunn 1990). Mountain whitefish (*Prosopium williamsoni*) in the mainstem Yakima River and rainbow trout in tributaries of the Yakima River also support fisheries. The varied population status and importance to humans result in differing acceptable impacts among these taxa. While acceptable impacts are based, in part, upon value judgments, it is clear that taxa whose abundance is low may not be able to withstand large impacts. Also, it should be clear that taxa that are highly valued by humans may produce fewer benefits if they sustained large impacts to their abundance. Impacts of management actions must be judged relative to the benefits of the action to determine what trade-offs are acceptable.

The status of a native salmonid population taxon can be characterized as the combination of distribution, abundance, and size structure. This study concentrates on detection of impacts to abundance, for simplicity. Abundance is the least equivocal evidence of an impact and has the potential of responding to the full range of interaction types (e.g., competition, predation, disease). These characteristics, in combination with standardization of methods due to common use, make abundance estimates an attractive candidate as a response variable for detecting ecological impacts to native salmonid species. Conversely, changes in abundance may take several years to become evident in surveys and may exhibit compensatory responses that limit early detection. To evaluate the potential for abundance monitoring to detect ecological impacts of a management action in time to implement corrective action, we needed to complete three major tasks. First, we conducted baseline monitoring to establish variability in the absence of the impact of interest. Second, we identified and modeled important biological and environmental factors to account for some of the interannual variation in population abundance. Finally, we estimated what size impact would be detectable for each taxon within five postimpact surveys. The sufficiency of detectable impacts could then be compared with acceptable impacts based upon the population status and human value of each taxa.

Baseline surveys

Our survey methods for resident taxa included boat and backpack electrofishing as well as snorkeling. Rainbow trout abundance was estimated annually, for a 91-km section of the mainstem of the upper Yakima River extending upstream from Roza dam, by mark–recapture methods in five index reaches averaging 5.5 km (Ham and Bartrand 1998). During rainbow trout surveys, mountain whitefish abundance was estimated by visual counts. In tributaries, annual surveys at 34 sites in 12 tributaries using multiple removal methods were used to estimate rainbow trout and cutthroat trout abundance. Bull trout abundance was estimated by intensive snorkel surveys at night (Pearsons et al. 1998). We have chosen to monitor anadromous species during downstream migration as smolts because potential impacts from the supplementation project should be more easily detected at the end of the freshwater rearing phase, before the influences of Columbia River migration and ocean rearing manifest themselves. Smolt data were collected by the Yakama Indian Nation, who have been counting anadromous salmon and steelhead at Chandler (smolts) and Prosser (adults) in the Yakima River basin for over 30 years (Major and Mighell 1969; Fast et al. 1991).

Modeling

To minimize unexplained variation in fish population abundance estimates, we attempted to account for some of the baseline temporal variation with environmental factors likely to influence abundance. Empirical regression models were constructed of the relationships between the estimated abundance of a native

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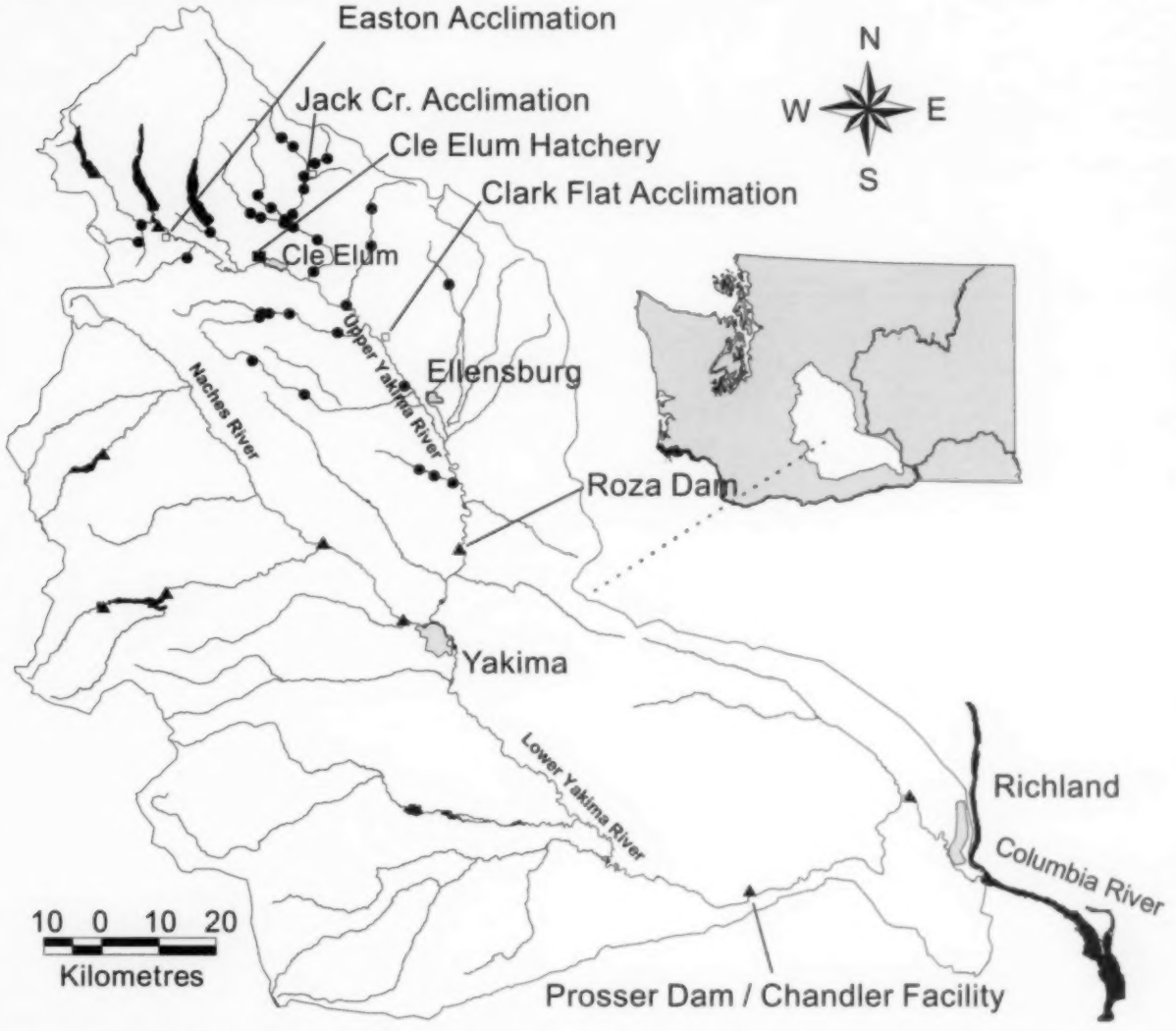
Fig. 1. Map of the Yakima River basin. The hatchery, acclimation sites, and counting or sampling locations for fish population abundance are illustrated. Resident fish are sampled intensively in the upper Yakima River basin, above Roza dam including mainstem and tributary index reaches. Circles indicate tributary sampling sites and triangles indicate dams. Anadromous fish are counted during emigration (as smolts) at the Chandler juvenile facility.



salmonid taxon and factors correlated with abundance. The predictive models were simple linear regression models of the form

$$\text{Predicted estimate} = \text{Intercept} + B_1(\text{index}_1) + B_2(\text{index}_2)$$

where B_1 through B_n are regression coefficients and index_1 through index_n are indices representing quantitative measures of sources of variation. The indices include factors such as water flows and temperatures, which are measured by the U.S. Bureau of Reclamation at many sites throughout the basin. Yearly variation in river conditions was summarized for each of several reporting stations along the Yakima River by computing the first two principal components of the group of monthly mean flows or temperatures. Factor scores of stations located in the range of a taxon were used as possible indices in the model. Models were chosen from the available indices for each taxa by stepwise multiple regression, with models limited to two or fewer indices to prevent overparameterization of the model, given the limited number of annual surveys available to generate the models. No attempt was made to construct a model for bull trout because only four baseline surveys had been conducted.

Power analyses and detectable impacts

Detectable impacts were computed for each taxon, given the baseline variation in estimates of abundance and models of other sources of variation. Testing for impacts is to be by a one-tailed t test of baseline abundance estimates versus abundance estimates during the potential impact period. We assumed that variance is proportional to mean abundance and therefore tested for differences in log-transformed abundance. Setting $\alpha = 0.1$ and $\beta = 0.1$ (power = 0.9), we computed what impacts would be detectable

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Table 1. Baseline population abundance and variation over time in the Yakima River basin.

Taxon	Units	Year										
		1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Bull trout	Number/km											
Cutthroat trout	Number/km								251	98	53	102
Fall chinook	Smolts (1000s)	104	44	68	33	154	76	28	111	55	253	149
Mountain whitefish	Number/km											276
Rainbow trout, mainstem	Number/km									131	117	129
Rainbow trout, tributary	Number/km								177	150	207	168
Spring chinook salmon	Smolts (1000s)	214	318	148	190	223	230	62	116	91	107	73
Steelhead	Smolts (1000s)	98	159	77	89	83	57	36	29	28	46	23

Table 2. Model parameters and variation explained by the model for three salmonid populations.

Taxon	Index 1	Index 2	Variation explained (%)
Cutthroat trout	Flow PC1 (lagged 1 year)	Flow PC1	42
Mountain whitefish	Flow PC1		48
Rainbow trout, mainstem	Flow PC1		49

Note: PC1 indicates the first principal component factor score.

with increasing numbers of postimpact surveys. The detectable impact can be computed with the following formula from Zar (1996, p. 135, eq. 8.23):

$$(1) \qquad \delta \geq \sqrt{\frac{2s_p^2}{n}}\,(t_{\alpha,\nu} + t_{\beta(1),\nu})$$

where n is the number of years to detect an impact, s_p^2 is the pooled sample variance, δ is the detectable impact, α is the significance level, ν is the degrees of freedom, df = $2(n - 1)$, β is the probability of a type II error (the power to detect an impact equal to δ is $1 - \beta$), $t_{\alpha,\nu}$ is the value from a one-tailed t table with probability α and ν df, and $t_{\beta(1),\nu}$ is the value from a one-tailed t table with probability β and ν df. The single value of n in eq. 1 assumes equal numbers of baseline and postimpact surveys. Because we are interested in the number of surveys required following the management action, it is necessary to rearrange eq. 8.21 in Zar (1996) to compute the value of n to use in eq. 1 as follows:

$$(2) \qquad n = \frac{2n_1n_2}{n_1 + n_2}$$

where n is the number of years to detect an impact calculated in eq. 1, n_1 is the number of baseline surveys completed before fish stocking begins, and n_2 is the number of postimpact surveys. Equation 2 corrects for the reduction in efficiency that is associated with unequal sample sizes in the baseline and potential impact periods. Equations 1 and 2 were used to plot detectable impact over a number of surveys following the onset of an impact. The plotted power relationships were compared among taxa to illustrate how temporal variation and the number of samples influence detectable impacts. To report detectable impacts as a percentage of the more familiar abundance scale, we computed the percent detectable impacts by dividing the antilog of the detectable difference by the baseline expected value and multiplying by 100.

We chose to limit detection time to minimize the potential for gradual changes in the environment over time to result in significant differences in abundance in the absence of the impact of interest. Climate cycles, anthropogenic changes to habitat or flow regimes, and pollution are some of the factors outside the test of supplementation that could alter fish abundance. To limit the influence of these types of changes within a test series, we have chosen to set a limit for detection of five annual surveys. That is, after 5 years of postimpact surveys have accumulated, only the five most recent surveys will be compared with the baseline mean. Longer or shorter environmental detection time limits might be more suited to the dynamics characteristic of other river systems

Results

Baseline surveys revealed high interannual variability for most taxa (Table 1). Coefficients of variation ranged from 26 to 94%. Attempts to explain that variability by modeling the influence of environmental influences was successful in some cases involving resident salmonids. Models were created for three of eight taxa, none of which were for anadromous taxa. Variation in flow accounted for between 42 and 49% of variation in abundance estimates for modeled taxa (Table 2). In-basin environmental conditions were not highly correlated with anadromous taxa abundance or tributary rainbow trout abundance, so models were not used.

Detectable impacts varied widely among native salmonid taxa. Figure 2 illustrates the relationship among the number of postimpact surveys and the detectable impact at a power level of 0.9 for each taxa, given the baseline level of temporal variation and the number of baseline surveys. This figure shows that increasing numbers of postimpact surveys beyond 5 years provides greatly diminishing returns, even under the assumption that environmental conditions and variation remain similar to the baseline period. For anadromous species, detection of impacts <47% (averaged across postimpact surveys) is unlikely, even after 10 surveys (Fig. 2*a*). For resident salmonids, detection of impacts <16% is unlikely, even after 10 surveys (Fig. 2*b*). Taxa with models applied, including mainstem rainbow trout, mountain whitefish, and cutthroat trout, have some of the lowest detectable impacts.

Detectable impacts with five postimpact surveys range from 19 to 79% of baseline abundance (Table 3). All anadromous taxa have detectable impacts above 50%, but among resident taxa, only bull trout has a detectable impact of >50%. Mainstem rainbow trout have the lowest estimated detectable impact at 19%.

Discussion

To answer the question that we posed in our title, "can reduced salmonid population abundance be detected in time to

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1994	1995	1996	1997	1998	*n*	Mean	CV
2.6			0.6	0.7	3	1.3	87
166	182	39	15	142	9	117	65
196	33	7	36	397	16	109	94
348	239	137	273	357	6	272	30
149	101	134	223	126	8	139	26
213	124	95	182	272	9	176	29
150	157	174	43	240	16	158	47
25	24	64	109	65	16	63	60

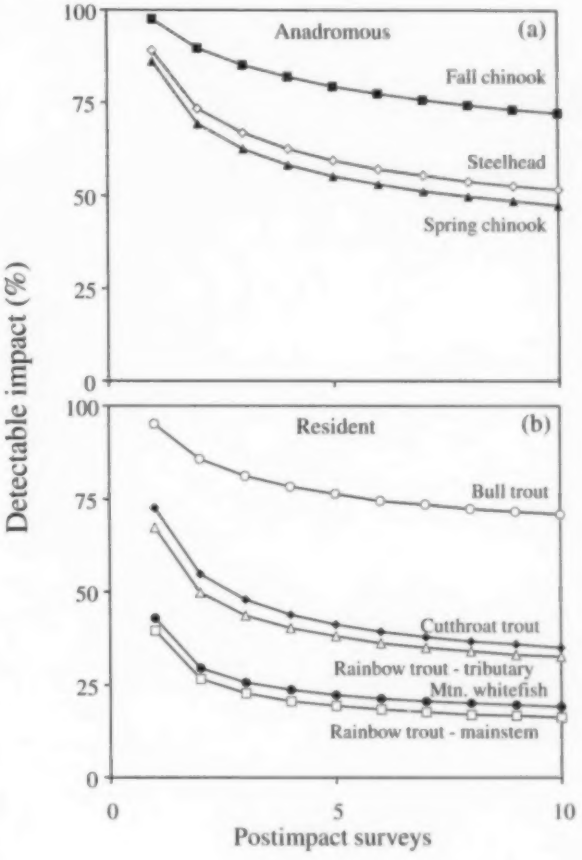
Table 3. Detectable impacts after 5 years expressed as a percentage of baseline abundance with $\alpha = 0.1$ and $\beta = 0.1$.

Taxon	Detectable impact (%)
Bull trout	76
Cutthroat trout	41
Fall chinook salmon	79
Mountain whitefish	22
Rainbow trout, mainstem	19
Rainbow trout, tributary	38
Spring chinook salmon	55
Steelhead	59

limit management impacts?," we must examine whether impacts can be detected in time to allow corrective actions to be implemented. We must also speculate what level of impact would be unacceptable, given the status and human value of a taxa and with consideration of the expected benefits of the management actions. Our results indicate that impacts to salmon population abundance <19% will not generally be detectable within 5 years (Table 3). Our results are consistent with studies of single populations that found that high temporal variation often makes detecting impacts difficult, even if longer times are available for detection (Van Winkle et al. 1981; Vaughan and Van Winkle 1982; Peterman and Bradford 1987). We suggest that impacts >19% would be unacceptable for species that are rare (e.g., species listed as threatened or endangered under the Endangered Species Act; bull trout, steelhead) or are highly valued (e.g., mainstem rainbow trout). Larger impacts might be acceptable to abundant less valued species, such as tributary rainbow trout or mountain whitefish, but it is the rare and highly valued taxa that are often the focus of protective efforts. Rapid, sensitive detection of impacts appears difficult to achieve through abundance monitoring. That conclusion leads us to question whether we might have achieved detection of much smaller impacts through better sampling or modeling.

To achieve rapid, sensitive detection of impacts to abundance, it would be necessary to achieve a very low unexplained variation. Unexplained variation can be reduced by reducing sampling error or by explaining variation with a model. The amount of variation that we observed in the Yakima basin baseline surveys was generally consistent with other studies of fish abundance (Fig. 3) (Grossman et al. 1990; House 1995; StreamNet 1998; Gibbs 2000). Figure 3

Fig. 2. Detectable impact versus number of postimpact survey years. The number of postimpact surveys is counted beginning at the first survey after the impact is imposed. Detectable impact computations assume a constant impact throughout the detection period.



indicates that four of eight of our populations exhibit variation equal to or greater than the median for other studies. For those taxa, there may be room for improvement in sampling. However, detectable impacts for our taxa that are at or below the lower range of variation found in other studies, such as mainstem rainbow trout, are still above 18%. Better sampling alone will not lead to detection of small impacts. High interannual variation does not indicate that rapid, sensitive impact detection is impossible if much of the variation can be explained by modeling the influence of other factors, such as flow variation.

The models we constructed did not explain enough variation to meet our goal of rapid, sensitive impact detection. Our models explained between 42 and 49% of the variation in abundance estimates. For comparison, we computed how much variation would need to be accounted for by a model to allow detection of a 5% impact. Our least variable anadromous species, spring chinook salmon, would require a model that accounted for 92% of variation to achieve detection of 5% impact at power = 0.9 within 5 years. The least variable resident salmonid, mainstem rainbow trout, would require a

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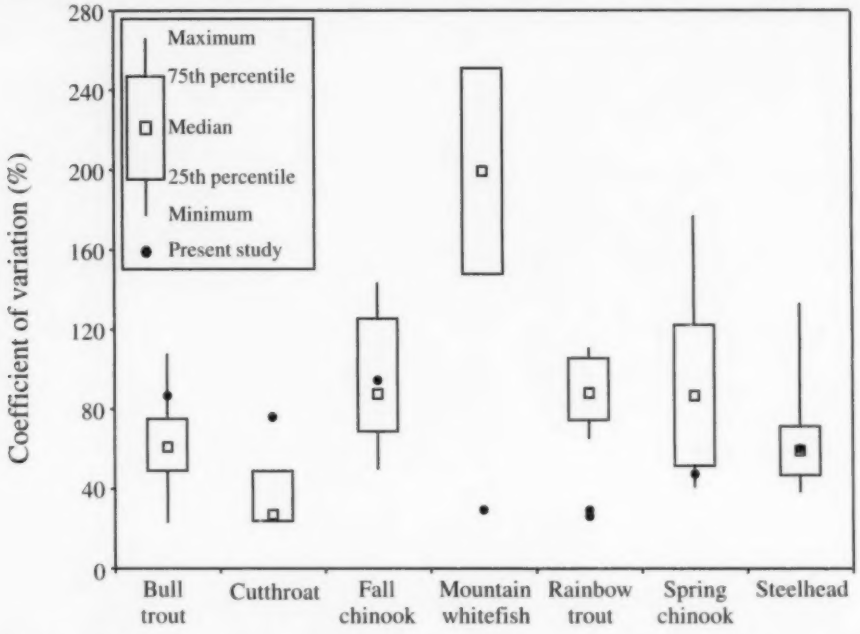
Fig. 3. Population abundance variation from this study versus previously published studies. Box and whisker diagram illustrates the range of variation in abundance of studies compiled from Grossman et al. (1990), House (1995), StreamNet (1998), and Gibbs (2000).



model that accounted for 76% of variation to achieve the same detection ability. Other taxa would require models that accounted for an even greater proportion of variation to achieve detection of 5% impact at power = 0.9 within 5 years. In predicting detectable impacts, we assume that neither the form nor the parameters of the model are wrong. If the model were wrong, our predicted ability to detect impacts would be biased and would likely be overly optimistic (Stewart-Oaten 1996). These scenarios suggest that a combination of low natural variability, low sampling error, and a good model could lead to rapid and sensitive detection of impacts. In our system, modeling is likely to be limited by the available information on influential factors because data may be lacking or collected on inappropriate time or spatial scales. These limitations may also obscure causal links between the management action and any impact that is detected. If the model fails to account for an important environmental influence, it could result in either over- or under-estimation of impacts. Generally, it seems unlikely that low variation and a very good model will occur in combination for other than a few well-studied and economically important species. For rare species, the likelihood of being able to make good estimates of abundance and create a good model seems more remote.

In spite of the limitations that we have demonstrated, abundance monitoring may serve a useful purpose in many impact assessments requiring less sensitive or less rapid detection. We have alluded to the possibility that, for some populations, large impacts may be acceptable and detection ability through abundance monitoring may be sufficient. If detection time is less critical, smaller impacts may be detectable at the desired level of power, making abundance monitoring a viable detection strategy. In the short term, abundance monitoring may fall short of detecting small impacts but may be the best available option for triggering a response to a large impact that is detectable. In other cases, abundance monitoring may provide secondary protection against impacts that may be missed by other monitoring options. Those wishing to use abundance monitoring to evaluate the consequences of management actions should not proceed without evaluating whether the predicted ability to detect impacts is sufficient for their application.

Conclusions

Monitoring the abundance of native salmonid populations is unlikely to result in detection of small impacts within a short period of time. The feedback required to alert managers in time to act and avert larger, possibly unacceptable, impacts to rare or highly valued fish taxa will be difficult, if not impossible, to achieve through abundance monitoring alone. Monitoring indicators such as size structure or specific interaction pathways may provide more rapid or sensitive impact detection than abundance monitoring and should be explored. Even if effective impact detection strategies can be implemented, we suggest that the risk assessment and uncertainty resolution process preceding monitoring efforts should be thorough. The results of the assessment would direct resources toward impacts of greatest concern or likelihood and alert managers to risks of impact that cannot be controlled through monitoring indicators of impact. With this information, managers or policy makers can make informed decisions to balance risks of a management action with its intended benefits.

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References

Bonneville Power Administration. 1996. Yakima fisheries project — final environmental impact statement. Bonneville Power Administration, Portland, Oreg. DOE/EIS-0169.

Busack, C., Watson, B., Pearsons, T., Knudsen, C., Phelps, S., and Johnston, M. 1997. Yakima fisheries project spring chinook supplementation monitoring plan. Bonneville Power Administration, Portland, Oreg. DOE/BP-64878-1.

Clune, T., and Dauble, D. 1991. The Yakima/Klickitat fisheries project: a strategy for supplementation of anadromous salmonids. Fisheries (Bethesda), **16**(5): 28–34.

Eberhardt, L.L., and Thomas, J.M. 1991. Designing environmental field studies. Ecol. Monogr. **61**: 53–73.

Fast, D.E., and Craig, C. 1997. Innovative hatchery project working to rebuild wild salmon populations. Hydro Rev. **14**: 30–33.

Fast, D., Hubble, J., Kohn, M., and Watson, B. 1991. Yakima River spring chinook salmon enhancement study. Final report submitted to Bonneville Power Administration, Portland, Oreg. DOE/BP-39461-9.

Gibbs, J.P. 2000. Monitoring populations. *In* Research techniques in animal ecology: controversies and consequences. *Edited by* T. Fuller and L. Boitani. Columbia University Press, New York. In press.

Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons, New York.

Grossman, G.D., Dowd, J.F., and Crawford, M. 1990. Assemblage stability in stream fishes: a review. Environ. Manage. **14**: 661–671.

Ham, K.D., and Bartrand, E.L. 1998. Salmonid distribution and population abundance variation in the upper Yakima River tributaries and implications for monitoring to detect impacts. *In* Yakima River species interactions studies. Progress report 1995–1997. *Prepared by* T.N. Pearsons, G.A. McMichael, K.D. Ham, E.L. Bartrand, A.L. Fritts, and C.W. Hopley. Bonneville Power Administration, Portland, Oreg. pp. 72–99.

House, R.A. 1995. Temporal variation in abundance of an isolated population of cutthroat trout in western Oregon, 1981–1991. N. Am. J. Fish. Manage. **15**: 33–41.

Hunn, E.S. 1990. Nch'i-Wana "The Big River" mid-Columbia Indians and their land. University of Washington Press, Seattle and London.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**: 1877–211.

Independent Scientific Group. 1996. Return to the river: restoration of salmonid fishes in the Columbia River ecosystem. Northwest Power Planning Council, Portland, Oreg.

Krause, T. 1991. The Yakima. Fly Fisherman, **22**: 40–43, 76–78.

Major, R.L., and Mighell, J.L. 1969. Egg-to-migrant survival of spring chinook salmon (*Oncorhynchus tshawytscha*) in the Yakima River, Washington. Fish. Bull. U.S. **67**: 347–359.

McMichael, G.A., Sharpe, C.S., and Pearsons, T.N. 1997. Effects of residual hatchery-reared steelhead on growth of wild rainbow trout and spring chinook salmon. Trans. Am. Fish. Soc. **126**: 230–239.

National Marine Fisheries Service. 1999. Endangered and threatened species: threatened status for two ESUs of steelhead in Washington and Oregon. Federal Register [Docket number 980225046-9070-03, 25 March 1999] **64**(57): 14 517–14 528.

National Research Council. 1995. Science and the Endangered Species Act. National Academy Press, Washington, D.C.

National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington, D.C.

Northwest Power Planning Council. 1994. Columbia Basin Fish and Wildlife Program. Adopted November 15, 1982; amended December 14, 1994. Northwest Power Planning Council, Portland, Oreg.

Pearsons, T.N. 1998. Draft objectives for non-target taxa of concern relative to supplementation of upper Yakima spring chinook salmon. *In* Yakima River species interactions studies. Progress report 1995–1997. *Prepared by* T.N. Pearsons, G.A. McMichael, K.D. Ham, E.L. Bartrand, A.L. Fritts, and C.W. Hopley. Bonneville Power Administration, Portland, Oreg. pp. 4–44.

Pearsons, T.N., McMichael, G.A., Ham, K.D., Bartrand, E.L., Fritts, A.L., and Hopley, C.W. 1998. Yakima River species interactions studies. Progress report 1995–1997. Bonneville Power Administration, Portland, Oreg.

Peterman, R.M. 1990. Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. **47**: 2–15.

Peterman, R.M., and Bradford, M.J. 1987. Statistical power of trends in fish abundance. Can. J. Fish. Aquat. Sci. **44**: 1879–1889.

Probasco, S. 1994. River journal. Vol. 2. Yakima River. Frank Amato Publications, Incorporated, Portland, Oreg.

Regional Assessment of Supplementation Planning. 1992. Supplementation in the Columbia Basin. Final report submitted to Bonneville Power Administration, Portland, Oreg. DOE/BP-01830-14.

Stewart-Oaten, A. 1996. Problems in the analysis of environmental monitoring data. *In* Detecting ecological impacts: concepts and applications in coastal habitats. *Edited by* R.J. Schmitt and C.W. Osenberg. Academic Press, New York. pp. 109–131.

Stewart-Oaten, A., Bence, J.R., and Osenberg, C.W. 1992. Assessing effects of unreplicated perturbations: no simple solutions. Ecology, **73**: 1396–1404.

StreamNet. 1998. StreamNet database (version 98.3), April 1998 (16 March 1998) (database downloaded to disk). StreamNet, Gladstone, Oreg. (URL: http://www.streamnet.org/accesstable.html).

U.S. Fish and Wildlife Service. 1998. Endangered and threatened wildlife and plants; determination of threatened status for the Klamath River and Columbia River distinct population segments of bull trout. Federal Register (June 10, 1998), **63**(111): 31 647 – 31 674.

Van Winkle, W., Vaughan, D.S., Barnthouse, L.W., and Kirk, B.L. 1981. An analysis of the ability to detect reductions in year-class strength of the Hudson River white perch (*Morone americana*) population. Can. J. Fish. Aquat. Sci. **38**: 627–632.

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Vaughan, D.S., and Van Winkle, W. 1982. Corrected analysis of the ability to detect reductions in year-class strength of the Hudson River white perch (*Morone americana*) population. Can. J. Fish. Aquat. Sci. **39**: 782–785.

Walters, C.J. 1986. Adaptive management of renewable resources. MacMillan, New York.

Walters, C.J., and Hilborn, R. 1976. Adaptive control of fishing systems. J. Fish. Res. Board Can. **33**: 145–159.

White, R.J., Karr, J.R., and Nehlsen, W. 1995. Better roles for fish stocking in aquatic resource management. Am. Fish. Soc. Symp. **15**: 527–547.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, N.J.

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Particulate light absorption and the prediction of phytoplankton biomass and planktonic metabolism in northeastern Spanish aquatic ecosystems

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Abstract: Examination of particulate light absorption and microplankton metabolism in 36 northeastern Spanish aquatic ecosystems, ranging from alpine rivers to inland saline lakes and the open Mediterranean Sea, revealed the existence of general relationships between particulate light absorption and the biomass of phytoplankton and microplankton metabolism. The particulate absorption spectra reflected a dominance of nonphotosynthetic, likely detrital, particles in rivers and a dominance of phytoplankton in coastal lagoons. There was a strong relationship between the light absorbed by phytoplankton and the chlorophyll *a* (Chl *a*) concentration of the systems, which indicated an average (±SE) Chl *a* specific absorption coefficient of 0.0233 ± 0.0020 m^2·mg Chl a^{-1} for these widely diverse systems. Chl *a* concentration was a weaker predictor of the total particulate light absorption coefficient, pointing to an important role of nonphytoplanktonic particles in light absorption. Gross production was very closely related to the light absorption coefficient of phytoplankton, whereas community respiration was strongly correlated with the total particulate light absorption coefficient, indicating the optical signatures of sestonic particles to be reliable predictors of planktonic biomass and metabolism in aquatic ecosystems.

Résumé : L'examen de l'absorption de la lumière par les particules et du métabolisme du microplancton dans 36 écosystèmes aquatiques du nord-est de l'Espagne, allant de rivières alpines à des lacs salins et à la Méditerranée, a montré l'existence de relations générales entre l'absorption de la lumière par les particules, la biomasse du phytoplancton et le métabolisme du microplancton. Le spectre d'absorption par les particules faisait état d'une dominance des particules non photosynthétiques, probablement d'origine détritique, dans les rivières et d'une dominance du phytoplancton dans les lagunes côtières. Il existait une importante relation entre la lumière absorbée par le phytoplancton et la concentration en chlorophylle *a* (Chl *a*) des systèmes. Le coefficient d'absorption spécifique moyen (±ET) de la Chl *a* était de 0,0233 ± 0,0020 m^2·mg Chl a^{-1} pour ces systèmes très différents. La concentration de Chl *a* était un moins bon indicateur du coefficient de l'absorption totale de lumière par les particules, ce qui porte à croire à une importante absorption de la lumière par les particules non phytoplanctoniques. La production brute présentait des rapports très étroits avec le coefficient d'absorption de la lumière du phytoplancton tandis que la respiration présentait une forte corrélation avec le coefficient d'absorption de la lumière des particules totales, montrant que les signatures optiques des particules sestoniques constituaient des indicateurs fiables de la biomasse et du métabolisme planctonique des écosystèmes aquatiques.

[Traduit par la Rédaction]

Introduction

Light absorption by suspended particles is an important characteristic of aquatic ecosystems because it greatly determines their optical properties and because it reflects the nature of these particles (Kirk 1983). In particular, light absorption by phytoplankton is an important component of planktonic primary production (Marra et al. 1993), but it is not necessarily the dominant source of light absorption by particles. Light absorption by other sestonic particles, including microheterotrophic organisms, can be quite substantial (e.g., Morel and Ahn 1990, 1991; Agustí 1994), as extensively demonstrated for marine waters (e.g., Iturriaga and Siegel 1989; Bricaud and Stramski 1990; Agustí and Cruzado 1992; Duarte et al. 1998). However, studies that examine the contribution of nonphytoplanktonic particles to particulate light absorption in freshwaters are still few, particularly in rivers, despite ample evidence that these particles are often present in relatively high concentrations in freshwaters, where they are known to have an important influence on vertical light extinction (Kirk 1983).

Most studies on underwater optics in freshwaters have been conducted with the aim of elucidating the mechanisms responsible for light attenuation, whereas studies addressing their possible use as predictors of planktonic processes are few and largely limited to the prediction of photosynthetic rates. The light absorbed by phytoplankton has been shown to be related to primary production (Marra et al. 1993), while the light absorbed by nonphytoplanktonic organisms has been postulated to be related to their biomass and metabolic activity (Agustí and Cruzado 1992; Agustí 1994). Be-

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cause the optical properties of phytoplankton and other sestonic particles do not necessarily differ between marine and freshwater systems, similar optical models may apply to predict sestonic biomass and metabolism across the full range of aquatic environments, as hypothesized earlier (Agustí and Cruzado 1992; Agustí 1994). If these hypotheses were confirmed, models predicting sestonic biomass and metabolism based on light absorption measurements could provide a unifying set of models of widespread application across aquatic ecosystems. There is therefore a need to test the existence of a general relationship between planktonic metabolism and light absorption by phytoplankton and nonphytoplanktonic particles applicable to a wide range of aquatic ecosystems, from alpine lakes and rivers to the upper ocean.

Here, we examine the hypothesized relationship between sestonic biomass and metabolism and light absorption by phytoplankton and nonphytoplanktonic particles in the widest range of aquatic ecosystems encountered in northeastern Spain. These range from high-mountain streams and lakes to endorheic saline lakes, coastal lagoons, marine bays, and the open Mediterranean Sea. We first examine the characteristic optical signatures of particles of the different types of aquatic ecosystems sampled and how they reflect phytoplankton biomass and the contribution of phytoplankton and nonphytoplanktonic particles to light absorption. We then describe planktonic community metabolism and test the hypothesized relationship between gross production and community respiration and particulate light absorption.

Methods

The 36 northeastern Spanish aquatic ecosystems studied include contrasting systems located across an elevation range from 0 to 2500 m above sea level (Table 1). They include rivers (11), from small high-mountain streams to the largest river in Spain (Ebro River), reservoirs (seven), small ponds (four) and a small lake (one), inland saline lakes (three), coastal lagoons (seven), and coastal (two) and open (one) Mediterranean waters. The sites were selected so as to provide the broadest possible diversity of conditions encountered in the region, thereby offering a robust test of the existence of general relationships between light absorption and sestonic biomass and metabolism consistent across contrasting ecosystems.

The systems studied were visited once between December 1993 and late May 1994. Surface waters were sampled and transported, with few exceptions, to the laboratory (within 1–6 h) for further analyses. These included estimation of the concentrations of chlorophyll *a* (Chl *a*) and evaluation of particulate light absorption and planktonic metabolism. A variable water volume (20–500 mL, depending on phytoplankton biomass) was filtered through Whatman GF/F filters for fluorometric analysis of Chl *a* concentration (Parsons et al. 1984). The filters were homogenized and kept refrigerated in the dark while pigments were extracted in 90% acetone for about 6 h. Fluorescence was measured, following extraction, in a Turner Designs fluorometer calibrated with pure Chl *a* (Sigma Chemical Co.).

Light absorption by suspended particles was measured on particles collected on filters, the method most widely used to measure light absorption of phytoplankton and sestonic particles, following Mitchell and Kiefer (1988). The measurement of optical density of particles collected in filters allows the estimation of the light absorption coefficient because the glassfiber filter diffuses light so that scattered light is still received by the photomultiplier. The path length amplification due to multiple scattering inside the filter needs to be corrected for (Bricaud and Stramski 1990). Seston was concentrated by filtering a variable volume of water (1–2.5 L, depending on suspended sediment concentration) through 2.5-cm Whatman GF/F filters. Light absorption by particles concentrated on the filters was measured immediately after collection. The optical density of the filters (OD_f) was measured in dual-beam scanning spectrophotometers (Shimadzu UV-2100 and JASCO-UV 7800) using a clean, water-saturated Whatman GF/F filter as a blank. The wet filters were placed in front of the photomultiplier and the clean, water-saturated filter used as a reference blank through baseline corrections. OD_f was measured at 1-nm intervals between 380 and 750 nm.

Particle light absorption (a_p, per metre) was calculated using the following the equation:

$$(1) \qquad a_p(\lambda) = 2.3 OD_f(\lambda) C/V \beta(\lambda)$$

where λ is the wavelength (nanometres), 2.3 is the factor to convert base 10 logarithms to natural logarithms, C is the clearance area of the filter (square metres), V is the volume of water filtered (cubic metres), and β is the wavelength-dependent path length amplification factor of the filters, calculated after Bricaud and Stramski (1990). The absorption attributable to phytoplankton (a_{ph}) and nonalgal particles (a_d) at the wavelengths measured was estimated from the sestonic absorption spectra ($a_p(\lambda)$) by an indirect method (Bricaud and Stramski 1990). This methods is based on the use of known ratios between light absorption by Chl *a* at peaks and valleys of its absorption spectra ($a_{ph}(505):a_{ph}(380) = 0.99$ and $a_{ph}(580):a_{ph}(692.5) = 0.92$) to deconvolute the bulk particulate light spectra into a phytoplankton and a nonalgal component (Bricaud and Stramski 1990). This method is semiempirical because it uses empirically derived relationships to approximate the absorption attributable to phytoplanktonic and nonphytoplanktonic particles from the total particulate absorption at specific wavelengths. The method of Bricaud and Stramski (1990) calculates the light absorption coefficient by nonalgal particles (a_d) as a negative exponential function of the wavelength, the parameters of which are derived as functions of the measured particulate light absorption spectra at 380, 505, 580, and 692.5 nm. The light absorption coefficient for phytoplankton ($a_{ph}(\lambda)$) is then calculated by subtracting the calculated absorption spectra of nonphytoplanktonic particles from the bulk particulate absorption spectra.

Particulate light absorption was summarized by the coefficients at 675 nm, an absorption peak of Chl *a*, to examine absorption by phytoplankton (e.g., Morel and Bricaud 1981). We also used the average light absorption between 400 and 700 nm (photosynthetically active radiation (PAR)) to examine the compound absorption of the different types of sestonic particles (algal and nonalgal particles). The absorption at 400 nm, where all suspended particles considered here absorb strongly, was also examined.

Water samples for the measurement of respiration and net production rates were filtered through a 150-μm mesh (to exclude larger zooplankton) and carefully siphoned into 125-mL narrow-mouthed Winkler bottles. Respiration and net production rates were calculated from changes in oxygen concentration after incubation of samples in the dark and in the light, respectively. Five replicates were used to determinate the initial oxygen concentration, and five replicate bottles were used for the incubations in the dark and in the light. Bottles were incubated at in situ temperature (range 5.5–18°C) for 15–24 h (Williams and Jenkinson 1982). "Light" bottles were incubated at 350 μmol photons·m^{-2}·s^{-1}. Dissolved oxygen concentration was measured using high-precision Winkler titration after Carrit and Carpenter (1966), using a Metrohm-682 autotitrator for the potentiometric (redox electrode) end-point detection (Oudot et al. 1988). The average coefficient of variation of the dissolved oxygen concentration was about 0.35%, and the resulting detection limit for net production and respiration

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Table 1. Particulate organic carbon (POC), Chl *a* concentration, dark respiration rates (DR), planktonic gross production rates (GP), and bulk particulate light absorption coefficients (a_p) at different wavelengths (PAR mean, 400 nm, and 675 nm) and the percentage of the particulat light absorption coefficient attributable to phytoplankton (% phyto) for the northeastern Spanish aquatic systems examined (—, not available).

System	Type	POC ($mg \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)	DR ($mg\ O_2 \cdot L^{-1} \cdot h^{-1}$)	GP ($mg\ O_2 \cdot L^{-1} \cdot h^{-1}$)	a_p(PAR) (m^{-1}) (% phyto)	a_p(675) (m^{-1}) (% phyto)	a_p(400) (m^{-1}) (% phyto)
Aigua Blava	Marine	0.29	1.36	0.0103	0.0069	0.014 (18.8)	0.008 (76.3)	0.037 (10.6)
Alfacs Bay	Marine	0.21	0.54	0.0179	0.0008	0.029 (20.9)	0.009 (75.6)	0.091 (9.9)
Arbucies	River	—	4.45	0.0122	0.0018	0.309 (11.2)	0.117 (62.2)	0.843 (5.5)
Breda	River	—	1.51	0.0126	0.0018	0.733 (0.0)	0.123 (46.0)	2.667 (0.3)
Bujaraloz	Pond	21.30	129.79	0.2154	1.4356	4.858 (86.2)	3.849 (99.8)	11.86 (60.1)
Muga Vella	Coastal lagoon	8.60	63.07	0.0667	0.4938	2.167 (90.5)	2.584 (99.8)	4.060 (70.2)
Chiprana	Saline lake	6.34	11.74	0.3045	0.2371	0.692 (45.6)	0.252 (86.8)	1.939 (26.3)
Embalse Pirenaico	Reservoir	—	1.70	0.0009	0.0071	0.062 (45.5)	0.046 (91.6)	0.158 (26.8)
Encañizada	Coastal lagoon	0.50	1.45	0.0231	0.0068	0.104 (20.9)	0.041 (75.5)	0.302 (9.7)
Estanca	Saline lake	0.19	5.05	0.0059	0.0317	0.204 (45.0)	0.089 (85.7)	0.514 (25.0)
Llausell	Reservoir	—	1.82	0.0002	0.0032	0.077 (35.4)	0.045 (86.8)	0.209 (19.9)
Massona	Coastal lagoon	0.98	11.13	0.0320	0.0507	0.383 (58.7)	0.305 (96.3)	0.931 (30.4)
Mequinenza	Reservoir	2.60	24.72	0.0167	0.2421	0.638 (47.5)	0.347 (90.8)	1.692 (27.0)
Mudstuck	Pond	9.12	142.15	0.0874	0.9236	1.720 (74.3)	1.901 (90.8)	3.405 (59.8)
Noguera	River	—	1.21	0.0026	0.0030	0.219 (0.2)	0.039 (24.6)	0.712 (0.6)
Open Sea	Marine	—	1.13	0.0099	0.0032	0.040 (32.8)	0.026 (83.5)	0.092 (14.6)
Pasteral	Reservoir	—	2.04	0.0045	0.0114	0.110 (51.3)	0.069 (91.3)	0.273 (32.7)
Pere Miquel Grande	Lake	—	5.56	0.0080	0.0218	0.317 (16.2)	0.114 (75.9)	0.996 (5.0)
Pere Miquel Pequeño	Pond	—	26.58	0.0232	0.1581	1.307 (14.2)	0.598 (72.9)	3.627 (5.0)
Platjola	Coastal lagoon	—	114.34	0.1376	2.4098	4.804 (82.6)	6.677 (98.7)	7.500 (60.7)
La Esparra	River	—	0.78	0.0160	0.0048	0.132 (3.5)	0.039 (47.7)	0.370 (0.1)
Sta. Coloma	River	—	15.45	0.0281	0.0690	0.407 (48.6)	0.274 (94.1)	1.146 (26.9)
Vila Ras	River	—	0.99	0.0025	0.0021	0.292 (2.8)	0.077 (58.9)	0.970 (0.2)
Ebro	River	0.54	7.17	0.0232	0.0668	0.315 (28.7)	0.153 (87.0)	0.993 (14.3)
Salardú	River	—	0.28	0.0008	0.0007	0.055 (0.2)	0.013 (44.2)	0.177 (0.3)
Santa Fé	Reservoir	0.88	16.07	0.0055	0.0346	0.251 (86.4)	0.206 (100)	0.678 (55.4)
Sta. Cristina	Pond	—	26.07	0.1056	0.3112	0.337 (70.7)	0.284 (95.6)	0.658 (51.2)
Susqueda	Reservoir	—	2.90	0.0050	0.0118	0.105 (47.4)	0.064 (89.7)	0.258 (28.5)
Tancada	Coastal lagoon	—	9.98	0.0237	0.0884	0.447 (21.8)	0.208 (83.3)	1.360 (7.1)
Taull	River	—	0.62	0.0006	0.0005	0.069 (2.8)	0.018 (53.6)	0.224 (0.2)
Tejo	Karstic lake	—	2.02	0.0011	0.0047	0.170 (46.3)	0.111 (80.2)	0.325 (34.3)
Ter	River	—	11.13	0.0108	0.1736	0.445 (45.3)	0.309 (93.3)	1.205 (22.3)
Ter Vell	Coastal lagoon	—	37.08	0.0957	0.3634	1.598 (69.4)	1.551 (96.6)	3.233 (47.4)
Tordera	River	—		0.0458	0.0809	0.387 (2.9)	0.106 (61.2)	1.251 (0.6)
Turies	Coastal lagoon	—	10.57	0.0302	0.0995	0.393 (83.7)	0.396 (99.6)	0.878 (59.1)
Uña	Reservoir	—	4.67	0.0030	0.0093	0.381 (28.7)	0.149 (70.9)	0.983 (19.3)
Mean ± SE	Rivers	0.54	4.03±1.5	0.01±0.004	0.029±0.015	0.51±0.09 (8.4±3.8)	0.32±0.05 (3.4±5.5)	1.16±0.23 (0.8±2.8)
	Reservoirs	1.74±0.85	7.7±3.4	0.0048±0.002	0.045±0.032	0.33±0.11 (34±6.1)	0.23±0.07 (50.5±4.9)	0.70±0.24 (25.5±4.4)
	Ponds and lakes	4.65±4.46	35.3±18.1	0.041±0.017	0.20±0.12	1.48±0.69 (35.5±13.7)	1.31±0.62 (48.6±13.9)	2.55±1.09 (27.4±11.9)
	Saline lakes	13.8±7.48	29.15±25.2	0.10±0.06	0.34±0.27	2.65±2.05 (42.2±9.8)	2.16±1.79 (52±8.1)	5.46±4.18 (34.3±8.9)
	Coastal lagoons	3.36±2.62	35.3±15.4	0.058±0.016	0.50±0.32	1.72±0.72 (47.2±10)	1.99±0.99 (63.9±9.7)	2.99±1.05 (35.9±8.7)
	Coastal and open sea	0.25±0.03	1.01±0.24	0.012±0.002	0.0036±0.0017	0.06±0.006 (13.2±4.3)	0.04±0.005 (28.8±8.5)	0.09±0.016 (8.6±1.6)

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was about 0.0009 mg $O_2 \cdot L^{-1} \cdot h^{-1}$. Respiration rates were calculated from the difference in oxygen concentration between initial and "dark" bottles, and net production was calculated from the change in oxygen concentration in "light" bottles. Because the day length varied considerably along the study, we based the comparison on instantaneous values, rather than on daily values. Gross primary production was calculated as the sum of community respiration rates and net community metabolism.

Results

The systems sampled ranged widely in phytoplankton biomass, as indicated by Chl *a* concentrations (Table 1). Particulate light absorption ranged greatly across systems, with the average absorption coefficient for the PAR range (400–700 nm) varying by three orders of magnitude (Table 1). In addition to the differences in magnitude, the shape of particulate light absorption differed greatly among ecosystems (Fig. 1). Rivers were characterized by an exponential decline in the particulate absorption coefficient with increasing wavelength, characteristic of nonphytoplanktonic particles (detritus, microheterotrophs, and inorganic particles) (Fig. 1). In contrast, coastal lagoons were characterized by particulate absorption coefficients closely dominated by photosynthetic pigments (Fig. 1), indicative of a dominance of phytoplankton in the seston of these waters. Other systems showed intermediate spectra with optical signatures reflecting a combination of phytoplankton and nonphytoplanktonic particles (Fig. 1). The absorption spectra were less variable in rivers and reservoirs than in other systems because rivers and reservoirs consistently showed spectra dominated by absorption by nonalgal particles (Fig. 1).

The particulate absorption coefficient was closely correlated ($r = 0.84$–0.88, $P < 0.0001$) with the Chl *a* concentration of the systems studied (Fig. 2), suggesting the differences in phytoplankton biomass to be an important source of differences in the particulate absorption coefficients. The relationship between Chl *a* concentration and the particulate absorption coefficients was strongest at 675 nm, an absorption peak of Chl *a*. Use of the deconvolution method to separate the absorption due to phytoplankton and other components of the suspended particle pool yielded much stronger correlations ($r = 0.91$–0.97, $P < 0.0001$) between Chl *a* concentration and the inferred light absorption by phytoplankton (Fig. 2). The strength of this correlation indicates that the specific light absorption coefficient is surprisingly similar among systems, with an average (±SE) of 0.0233 ± 0.0020 $m^2 \cdot mg\ Chl\ a^{-1}$ (range 0.0045–0.0576 $m^2 \cdot mg\ Chl\ a^{-1}$), despite the very wide range of aquatic systems examined.

The significant relationships between Chl *a* concentration and the particulate light absorption coefficients measured in the systems studied do not necessarily imply that phytoplankton are the main contributor to particulate light absorption. Examination of the percentage of the particulate light absorption contributed by phytoplankton (Table 1) reveals major differences among systems and among wavelengths (Fig. 3). The contribution of phytoplankton to particulate light absorption is greatest (generally >70%) at 675 nm, an absorption peak of Chl *a*, and is lowest (generally <30%) at 400 nm, where all components of the seston, living or not, absorb strongly, adopting intermediate values (around 50%) for the mean absorption coefficient across the PAR range (Fig. 3). The contribution of phytoplankton to particulate light absorption across the PAR band varied significantly across system types (ANOVA, $F = 4.6$, $P < 0.01$), with rivers, reservoirs, and marine systems showing the smallest contribution of phytoplankton and coastal lagoons and saline lakes showing the greatest contribution of phytoplankton (Fig. 1).

Community metabolism also varied greatly among systems, with rivers, reservoirs, and marine systems tending to support lower gross production rates (Fig. 4). Differences in community metabolism among systems generally followed those in gross production, suggesting a general correlation between community production and respiration (Fig. 5), except for marine planktonic communities, which tended to show relatively higher community respiration rates (Fig. 4). Both gross production and community respiration rates were significantly correlated with the Chl *a* concentration of the systems, although the correlation was weaker for respiration rate ($r = 0.75$ versus $r = 0.95$ for gross production), for which Chl *a* concentration is not such strong a predictor (Fig. 6).

The weaker correlation between Chl *a* concentration and community respiration indicates that phytoplankton are, on average, unlikely to be a dominant component of the respiration of the planktonic communities studied. This suggests an important role of other planktonic organisms in the metabolism of planktonic communities, consistent with their importance for light absorption. The light absorption coefficient of phytoplankton at 675 nm ($a_{ph}(675)$) was closely related to the gross community production (Fig. 7), as described by the regression equation

$$\log \text{gross production (mg } O_2 \cdot L^{-1} \cdot h^{-1}) = -0.53 + 1.12\ (\pm 0.09) \log a_{ph}(675)\ (m^{-1}),$$
$$R^2 = 0.80,\ SE_{\log \text{ estimate}} = 0.43,$$
$$F = 139,\ P < 0.00001$$

which indicates that gross production increases linearly (H_0: slope = 1, *t* test, $P > 0.05$) with increasing light absorption by phytoplankton. The strength of the relationship suggests that community gross production can be predicted from the light absorption coefficient of phytoplankton with a twofold prediction error, as indicated by the standard error of the estimates, which is quite satisfactory given the very diverse nature of the systems studied. Although this precision is better than the order-of-magnitude precision characteristic of comparative approaches, the twofold prediction error does not allow for strong inferences when comparing similar systems.

The community respiration rate was, however, best correlated with the average particulate absorption coefficient over the PAR band (a_p(PAR), Fig. 7), as described by the regression equation

$$\log \text{community respiration (mg } O_2 \cdot L^{-1} \cdot h^{-1}) = -1.43 + 0.71\ (\pm 0.16) \log a_p(\text{PAR})\ (m^{-1}),$$
$$R^2 = 0.39,\ SE_{\log \text{ estimate}} = 0.52,$$
$$F = 20.2,\ P = 0.0001$$

which indicates that community respiration changes as the 3/4 power of the average particulate light absorption over the

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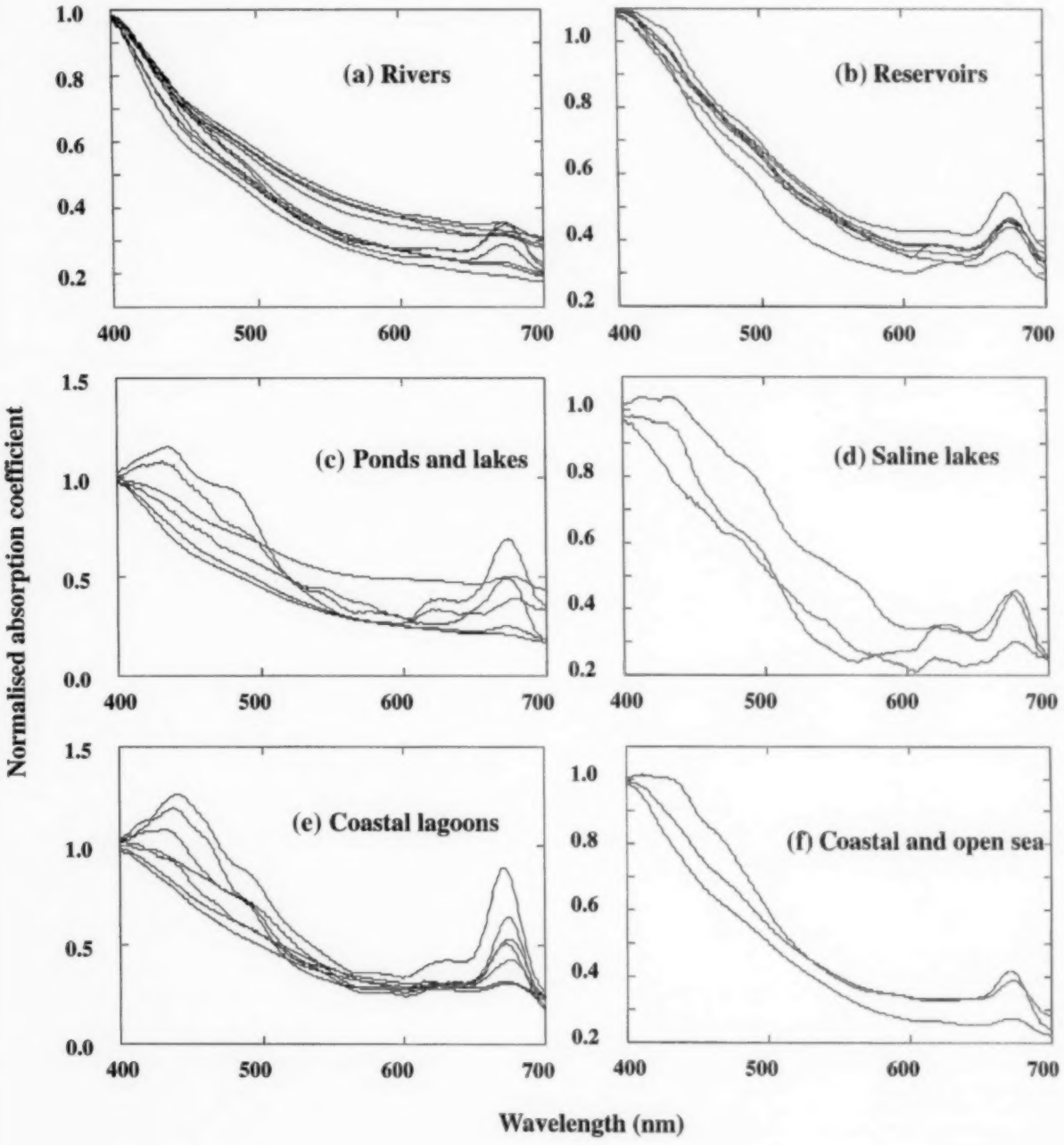
Fig. 1. Normalised particulate absorption coefficients for the different types of northeastern Spanish aquatic systems sampled. The spectra were normalised by dividing the coefficients by the absorption at 400 nm, allowing the comparison of their shapes.



PAR range. The relationship between community respiration rate and particulate light absorption has a predictive potential similar to that between gross production and phytoplankton absorption, as indicated by the similar standard error of the estimates. However, due to the lower range of variation in community respiration rate, the relationship appears to be more scattered than that for gross production (Fig. 7). There was a tendency for community respiration to be somewhat lower in rivers and reservoirs relative to marine systems with similar particulate light absorption coeffi-

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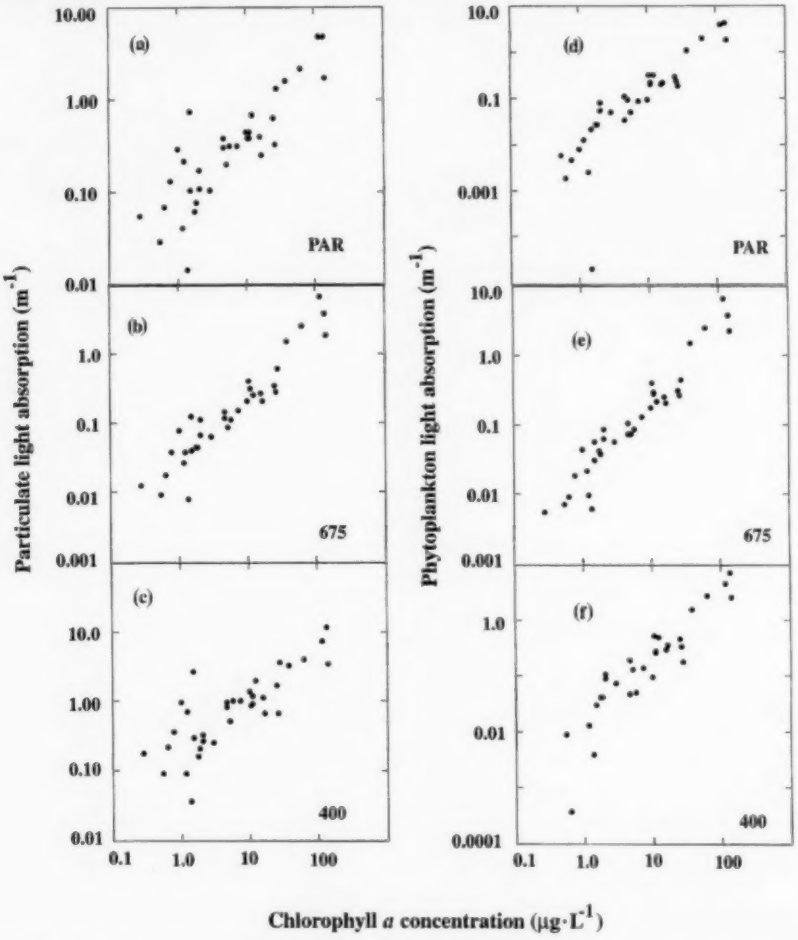
Fig. 2. Relationship between Chl *a* concentration of northeastern Spanish aquatic systems and the total particulate absorption coefficient and that of phytoplankton at different wavelengths.



cients. This tendency likely reflects a greater contribution of the nonliving component of the seston to particulate light absorption in rivers and reservoirs compared with marine waters.

Discussion

The demonstration of a strong relationship between Chl *a* concentration and particle optics across the diverse systems examined supports the hypothesis of the existence of a universal relationship for aquatic ecosystems. Moreover, the Chl *a* specific absorption coefficient of phytoplankton is remarkably uniform across the broad spectrum of aquatic ecosystems examined. This uniformity suggests that the broad variability in optical properties of individual phytoplankton species is averaged out in the multispecific communities represented in the natural plankton, leading to remarkable conservative Chl *a* specific absorption coefficients in phytoplankton communities. The findings reported are consistent with the absence of substantial differences, in terms of optical properties, between the particles present in different aquatic ecosystems, so that the processes determining particulate light absorption are the same across systems. The relative contribution of different particle types to the suspended solids pool, and therefore the bulk particulate light absorption, does change substantially, however. In particular, suspended solids in rivers appear to be dominated by detrital, likely nonliving, particles, consistent with the importance of allocthonous inputs to these systems and the high turbulence able to keep them in suspension there, those in coastal lagoons by phytoplankton cells, and those in marine systems by heterotrophic microorganisms (cf. Agustí and Cruzado 1992; Gasol et al. 1997). These differences in the composition of the pool of suspended particles result in spectral differences in particulate light absorption among systems (Fig. 1) and substantial variability in the relative contributions of phytoplankton and other particles to total particulate light absorption (cf. Duarte et al. 1998).

The results obtained support the hypothesis that the optical signature of the particles reflected in the bulk light absorption spectrum is not only indicative of the nature of the particles present but also of the metabolism of the communi-

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Fig. 3. Box plots showing the distribution of the percent light absorbed by phytoplankton at different wavelengths in the northeastern Spanish aquatic systems sampled. Boxes encompass the 25 and 75% quartiles, the central line represents the median, and the bars encompass 95% of the values. Asterisks indicate observations outside the 95% limits.

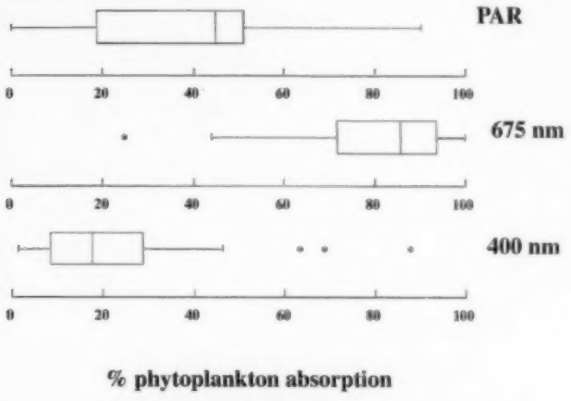


Fig. 4. Box plots showing the distribution of (*a*) gross production and (*b*) community respiration in the different types of northeastern Spanish aquatic systems sampled. Boxes designed as in Fig. 3.

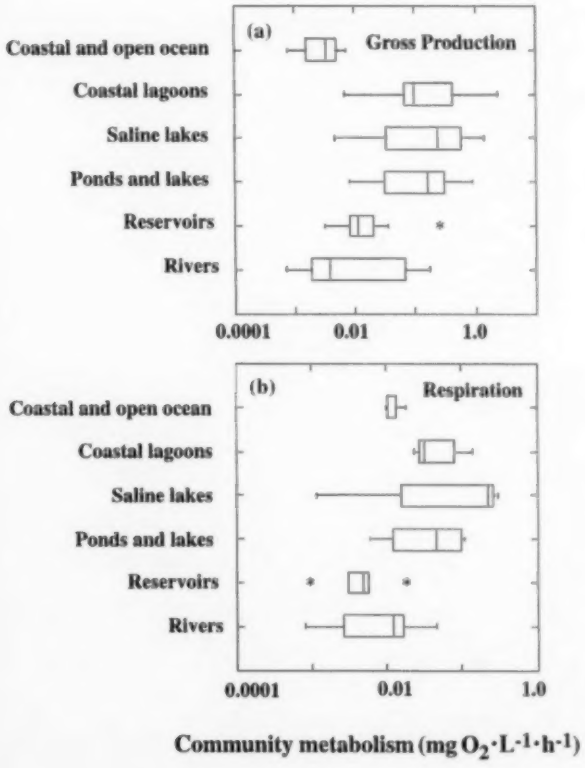
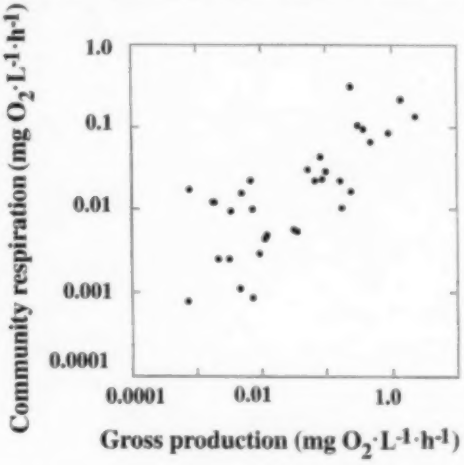


Fig. 5. Relationship between community respiration and gross production in the northeastern Spanish aquatic systems sampled.



ties (cf. Agustí and Cruzado 1992; Agustí 1994). The gross production rate is linearly scaled to light absorption by phytoplankton, as previously reported by Marra et al. (1993), with a similar relationship holding for diverse types of aquatic ecosystems. However, community respiration is also closely related to the average bulk particulate absorption coefficient over the PAR range. While the relationship between light absorption by phytoplankton and gross production is well supported by the mechanisms of photosynthesis, the mechanistic basis for the relationship between particulate light absorption and community metabolism is far less clear. Both autotrophic and heterotrophic organisms contribute to community respiration and particulate light absorption. Data on light absorption by heterotrophic planktonic organisms are still sparse, but available information shows similar light absorption spectra for micro- and mesoheterotrophs characterized by high absorption in the blue region and an exponential decline towards the red band (e.g., Morel and Ahn 1990, 1991; Agustí 1994). Light absorption by respiratory pigments at about 410 nm (Morel and Ahn 1991) provides a mechanistic link between light absorption by heterotrophs and respiration rate, although light absorption by respiratory pigments is likely to be a minor component of the light absorbed by heterotrophs. The relationships between planktonic metabolism and particulate absorption are remarkably strong considering the broad range of temperature (5–18°C) in the systems and that must certainly influence metabolic, particularly respiration, rates. Future efforts to parametrize the temperature dependence of planktonic metabolism may lead, when combined with the particulate absorption parameters used here, to improvement of the predictive power of the relationships described.

The contrasting importance of algal and nonalgal particles in light absorption across the systems studied is consistent with the balance between production and respiration in these ecosystems (Duarte and Agustí 1998). Rivers tend to be strongly heterotrophic (Duarte and Agustí 1998), and their seston is likely to be dominated by nonalgal particles, as observed here. In contrast, the seston of eutrophic lakes is dominated by phytoplankton, and these productive systems

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Fig. 6. Relationship between (*a*) gross production and (*b*) community respiration and the Chl *a* concentration of the northeastern Spanish aquatic systems sampled.

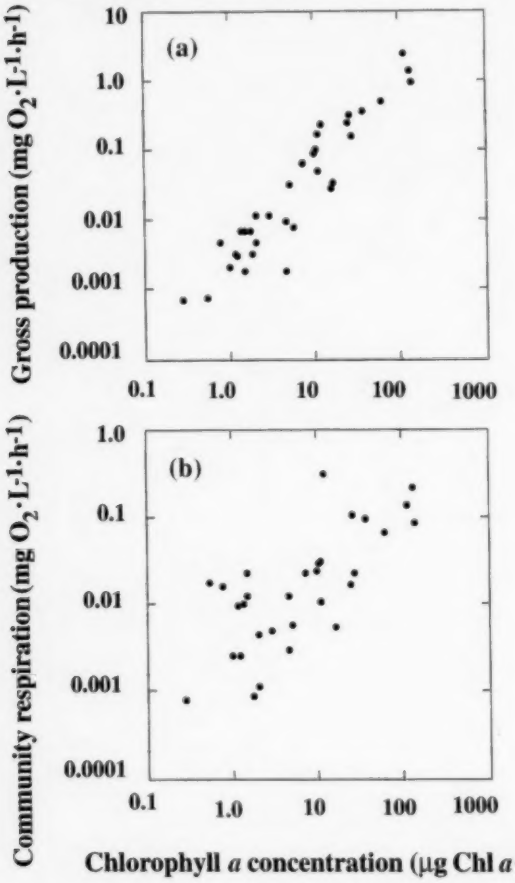
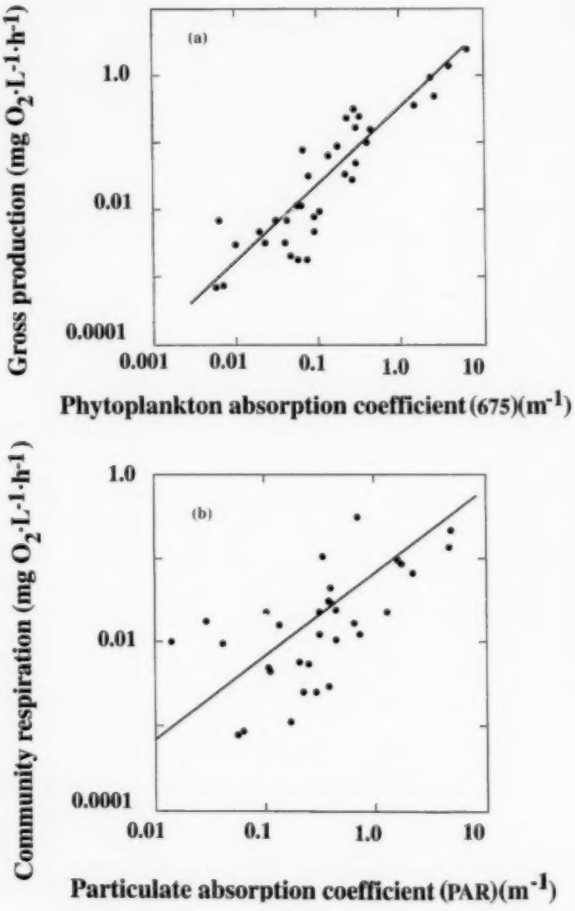


Fig. 7. Relationship between (*a*) gross production and (*b*) community respiration and the absorption coefficient of phytoplankton and the total particulate absorption coefficient of the northeastern Spanish aquatic systems sampled. Lines represent the fitted regression equations.



tend to be autotrophic (Duarte and Agustí 1998). Lastly, the plankton of unproductive marine systems, such as the Mediterranean Sea, tend to be heterotrophic (Duarte and Agustí 1998), consistent with the dominace of heterotrophic organisms in these planktonic communities (Gasol et al. 1997). Hence, the results reported for northeastern Spanish aquatic ecosystems conform to general patterns in metabolism and seston composition of aquatic ecosystems and should therefore be applicable to aquatic ecosystems elsewhere.

The results presented confirm the existence of a general relationship between particulate light absorption and phytoplankton biomass and community metabolism applicable to a broad spectrum of aquatic ecosystems. The precision of the empirical relationships derived is such (twofold estimate error) that they hold potential for the prediction of system metabolism. Their strength provides evidence of the importance of the information contained in optical signatures of sestonic particles and further demonstrates the usefulness of biooptical models as powerful tools to model planktonic processes. Moreover, the applicability of the models reported here to a wide array of aquatic ecosystems shows that bio-optical models have general application in aquatic ecology and provide unifying tools, helping to bridge the present gap between research programs focused on different types of aquatic ecosystems.

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References

Agustí, S. 1994. Planktonic size structure and the photon budget of the euphotic ocean. *In* The size structure and metabolism of the pelagic ecosystem. *Edited by* J. Rodríguez and W.K.W. Li. Sci. Mar. **58**: 109–117.

Agustí, S., and Cruzado, A. 1992. Relationship between light absorption by pelagic particles and microplankton metabolic activity in the Gulf of Lions. Mar. Ecol. Prog. Ser. **85**: 283–287.

Bricaud, A., and Stramski, D. 1990. Spectral absorption coefficients of living phytoplankton and non-algal biogenous matter: a comparison between the Peru upwelling area and the Sargasso Sea. Limnol. Oceanogr. **35**: 562–582.

Carrit, D.E., and Carpenter, J.H. 1966. Comparison and evaluation of currently employed modifications of the Winkler methods for determinating dissolved oxygen in sea water. A NASCO report. J. Mar. Res. **24**: 286–318.

Duarte, C.M., and Agustí, S. 1998. The CO_2 balance of unproductive aquatic ecosystems. Science (Washington, D.C.), **281**: 234–236.

Duarte, C.M., Agustí, S., Satta, M.P., and Vaqué, D. 1998. Partitioning particulate light absorption: a budget for a Mediterranean Bay. Limnol. Oceanogr. **43**: 236–244.

Gasol, J.M., del Giorgio, P.D., and Duarte, C.M. 1997. Biomass distribution in marine planktonic communities. Limnol. Oceanogr. **42**: 1353–1363.

Iturriaga, R,. and Siegel, D.A. 1989. Microphotometric characterisation of phytoplankton and detrital absorption properties in the Sargasso Sea. Limnol. Oceanogr. **34**: 1706–1726.

Kirk, J.T.O. 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, U.K.

Marra, J., Chamberlin, W.S., and Knudson, C. 1993. Proportionality between in situ carbon assimilation and bio-optical measures of primary production in the Gulf of Maine in summer. Limnol. Oceanogr. **38**: 232–238.

Mitchell, B.G., and Kiefer, D.A. 1988. Chlorophyll *a* specific absorption and fluorescence excitation spectra for phytoplankton. Deep-Sea Res. **35**: 639–663.

Morel, A., and Ahn, Y.-H. 1990. Optical efficiency factors of free-living bacteria: influence of bacterioplankton upon the optical properties and particulate organic carbon in oceanic waters. J. Mar. Res. **48**: 145–175.

Morel, A., and Ahn, Y.-H. 1991. Optics of heterotrophic nanoflagellates and ciliates: a tentative assessment of their scattering role in oceanic waters compared to those of bacterial and algal cells. J. Mar. Res. **49**: 177–202.

Morel, A., and Bricaud, A. 1981. Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep-Sea Res. **28**: 1375–1393.

Oudot, C., Gerard, R., Morin, P., and Gningue, I. 1988. Precise shipboard detrmination of total dissolved oxygen (Winkler procedure) for productivity studies with a commercial system. Limnol. Oceanogr. **33**: 146–150.

Parsons, T.R., Maita, Y., and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Tarrytown, New York.

Williams, P.J. leB., and Jenkinson, N.W. 1982. A transportable microprocessor-controlled Winkler titration suitable for field and shipboard use. Limnol. Oceanogr. **27**: 576–584.

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Metallothionein-like proteins in *Macoma balthica*: effects of metal exposure and natural factors

C. Mouneyrac, A. Geffard, J.C. Amiard, and C. Amiard-Triquet

Abstract: Biological processes involved in the tolerance acquired by populations chronically exposed to metal pollution in the environment were examined in baltic clams (*Macoma balthica*) originating from both industrialized and clean areas and in clams exposed experimentally to metals. It has been shown previously that clams surviving Ag and Hg exposure at LT50 did not protect themselves by accumulating smaller amounts of metals than clams that failed to survive, so attention was focussed on the physicochemical forms of storage of the accumulated metals. Silver was found to be predominantly bound to insoluble forms and Cd and Hg to soluble forms. In both controls and contaminated clams, a metallothionein-like protein (MTLP) has been shown to be present, the concentrations of which did not depend on the geographical origin of the clams. The significant relationship between metal and MTLP concentrations shown in the baltic clams suggests that the induction of this protein could provide a useful tool for the biomonitoring of metal pollution. The influence of natural factors (season, weight), however, must be taken into account when interpreting such data.

Résumé : Les processus biologiques impliqués dans la tolérance acquise dans les populations exposées de façon chronique aux métaux dans leur milieu ont été examinés chez *Macoma balthica* provenant de sites industrialisé ou naturel et contaminées par trois métaux. Il a été montré antérieurement que ces bivalves, lorqu'ils survivent à la CL50 d'Ag ou Hg, ne se sont pas protégés en accumulant moins de métaux que ceux qui meurent, aussi le rôle des formes physicochimiques de stockage était à évaluer. L'argent était stocké majoritairement sous forme insoluble et Cd et Zn sous forme soluble. Chez les individus témoins et contaminés, une protéine type métallothionéine (MTLP) était présente, à des niveaux indépendants de l'origine géographique. La relation entre concentrations de métaux et de MTLP chez *Macoma balthica* suggère que l'induction de cette protéine pourrait être utilisée pour la biosurveillance des pollutions métalliques. Cependant, l'influence des facteurs naturels (saison, poids) doit être prise en compte pour interpréter les résultats.

Introduction

Tolerance to metal pollution has been shown to occur in different species chronically exposed to metal pollutants in their environment (Klerks and Weis 1987). Organisms exposed during some period of their life cycle may develop a physiological acclimation. Metallothionein (MT) is a cysteine-rich, heat-stable, low molecular weight protein that binds metals such as Ag, Cd, Cu, Hg, and Zn and thus contributes to metal detoxication. The synthesis of MT is often induced when organisms are exposed to environments contaminated by heavy metals. Thus, induction of MT by metals in aquatic organisms could provide a useful tool for biological monitoring of metal pollution, bivalves such as *Mytilus edulis* (George and Olsson 1994) and *Crassostrea gigas* (Mouneyrac et al. 1998) being the most promising candidates among invertebrates.

In numerous studies, analytical procedures have been based on several characteristics of MTs that do not strictly allow the assertion that the studied molecule is a true MT primarily because purification and sequencing have not been carried out. The terminology of metallothionein-like protein (MTLP) will be used in these cases.

In *Macoma balthica*, an intrasedimentary bivalve widely distributed in marine and estuarine habitats, any detoxication involving MT was apparently not present in individuals originating from southern England and exposed to Cd (Langston and Zhou 1987). On the other hand, however, the possible role of a MTLP binding Ag and Cu has been indicated in Pacific American specimens of *M. balthica* (Bray et al. 1983; Johansson et al. 1986). Similarly, in *M. balthica* originating from the estuary of the western Scheldt, exposure to Cd, Cu, and Zn induced the increase of cytosolic concentrations of metals and metal-binding proteins displaying several of the main characteristics of MTs (Bordin et al. 1994, 1997). Again, in individuals from the Loire estuary (France), Rigaa et al. (1998) showed the presence of a Cd-inducible protein exhibiting some characteristics of MTs. In *M. balthica* collected from several Arctic estuaries, cytosolic heat-stable compounds were demonstrated using the

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Table 1. List of the different groups of *M. balthica* analyzed for metals and MTLPs in the present study.

Site	Season	Weight-class	Mean (SD) wet weight of soft tissues (g)	Experimental exposure (ng metal·mL^{-1})
Loire	January	Medium	0.029 (0.072)	Control; Hg 100
	April	Large	0.541 (0.116)	Control; Ag 10; Cd 10
		Medium	0.287 (0.119)	Control; Ag 10, 50, and 100; Cd 10; Hg 100
		Small	0.109 (0.008)	Control; Ag 10; Cd 10
	October	Large	0.747 (0.099)	Control; Ag 10; Cd 10
		Medium	0.387 (0.061)	Control; Ag 10; Cd 10
Somme	January	Medium	0.241 (0.062)	Control
	April	Medium	0.393 (0.080)	Control; Ag 50; 100; Hg 100

polarographic assay, suggesting the presence of MTLP, the concentration of which varied among stations according to Cu contamination in sediments and soft tissues of bivalves (Amiard-Triquet et al. 1998). MT concentrations in tissues are often highly variable and remarkably susceptible to the influence of a large variety of endogenous and exogenous factors (e.g., weight, season) (George and Olsson 1994; Amiard and Cosson 1997; Mouneyrac et al. 1998). In *M. balthica*, weight has also been shown to influence MTLP concentrations (Amiard-Triquet et al. 1998). Body weight fluctuations associated with reproduction were probably responsible for the seasonal changes observed in baltic clams from the estuary of the western Scheldt (Bordin et al. 1997).

When considering MT as a biomarker of metal pollution, it is necessary to determine if any change in MT level after metal exposure is higher than MT level changes resulting from factors not connected to metal exposure. Thus, in the present study, we examined the influence of natural factors (weight, season) concurrently with that of metal exposure (Ag, Cd, Hg) in *M. balthica*. In order to take into account the tolerance potentially acquired by populations chronically exposed to metal pollution in their environment, specimens originating from clean and industrialized areas were used in the present study.

Materials and methods

Specimens

Macoma balthica were collected in January, April, and October 1996 from two estuaries (Loire and Somme). According to the data of the French Mussel Watch (Réseau National d'Observation 1995), concentrations of Hg, Cd, Cu, Pb, and Zn in mussels originating from the industrialized Loire estuary are higher than in those from the Bay of Somme, which is a nature reserve (Parc du Marquenterre). In the laboratory, the animals were acclimated in seawater (salinity 32‰) for 1 week prior to the experiments. To exclude any effect of temperature as a stressor agent, the temperatures of aquaria were identical to those of natural waters (12–14°C). Subsequently, groups of 20 individuals were held in aquaria containing 2 L of filtered (1 µm) seawater, salinity 32‰, and exposed to Cd (10 ng·mL^{-1}), Ag (10, 50, and 100 ng·mL^{-1}) or Hg (100 ng·mL^{-1}). Control groups were held under the same conditions. Water in each tank was not aerated and was changed every other day. Mortality was recorded daily and the experiments continued until about 50% mortality of the clams had been observed. The mean lethal times (LT50) were determined by the probit method (Bliss 1938). In clams exposed to 100 ng Hg·mL^{-1}, the LT50 was equal to 8.0 and 8.7 days, respectively, for specimens originating from the Loire and Somme estuaries in April and reached 12.2 days in January in Loire samples. In clams exposed to 50 or 100 ng Ag·mL^{-1} in April, the LT50 was 8.6 and 5.6 days, respectively, in Loire samples and 10.4 and 4.5 days in Somme samples. By contrast, for controls and *M. balthica* exposed to Cd or Ag at 10 ng·mL^{-1}, no mortality was observed, and in these cases, exposure lasted 10 days. At the end of each experiment, survivors were frozen at –80°C until analysis. All groups of samples are shown in Table 1.

Condition

The shell length of each clam was measured. Whole soft tissues were removed from the shells and weighed. The condition index was calculated for all animals individually as the weight per volume assessed from (length)^3.

Compartmentalization

Soft tissues of individual clams (large and medium weight-classes, see Table 1) or pools of five specimens (small weight-class) were homogeneized in a solution of 20 mM Tris, 10^{-5} mM β-mercaptoethanol, and 150 mM NaCl adjusted to pH 8.6 (4 mL·g^{-1} soft tissue). The soluble (S1) and insoluble (P1) fractions were separated by centrifugation (25 000 × *g* for 55 min). The soluble heat-stable compounds including MTLP were isolated by centrifugation of the S1 fraction (12 000 × *g* for 10 min) after heat treatment (75°C for 10 min).

MTLP analysis

In the heat-denatured cytosol (S2), the amount of MTLP was determined by differential pulse polarographic analysis, a technique based on measurement of –SH compounds, as described in Thompson and Cosson (1984). A PAR model 174 analyzer, a PAR/EG&G model 303 static mercury drop electrode, and an X-Y recorder (RE 0089) were used. The temperature of the cell was maintained at 5°C. The standard addition method was used for calibration with rabbit liver MT (Sigma Chemical Co., St. Louis, Mo.) in the absence of purified bivalve MT. Results are expressed as milligrams of MTLP per gram wet weight of homogenized tissue.

Metal analysis

The determination of trace elements was performed with extreme caution to avoid secondary contamination. All labware was soaked in 10% hydrochloric acid, rinsed three times with deionized water, and dried in a desiccator sheltered from atmospheric dust. Only high-quality reagents were used and the pretreatment of specimens devoted to Hg determination was carried out with reagents labelled as "special grade for the determination of mercury." The P1 and S1 fractions were heated (65°C for 12 h) with suprapure nitric acid (1 mL) and hydrochloric acid (1 mL). After digestion, Ag and Cd levels in these acid solutions were determined, after dilution with deionized water, by flameless atomic absorption spectrophotometry using the Zeeman effect. The analytical method

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Fig. 1. Relationship between soluble (S1, ○) or insoluble (P1, ▲) Ag and total Ag (S1 + P1) in the soft tissues of *M. balthica* (ng Ag·g wet weight^{-1}) exposed to 10, 50, and 100 ng Ag·mL^{-1} or controls.

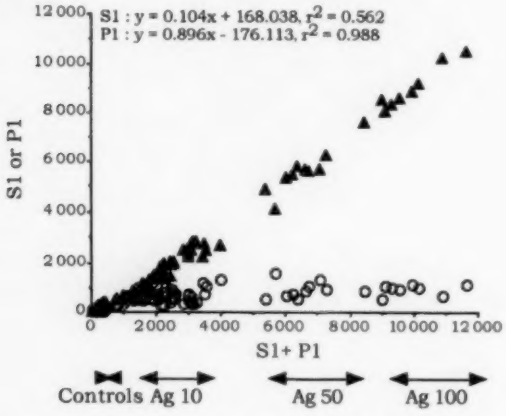
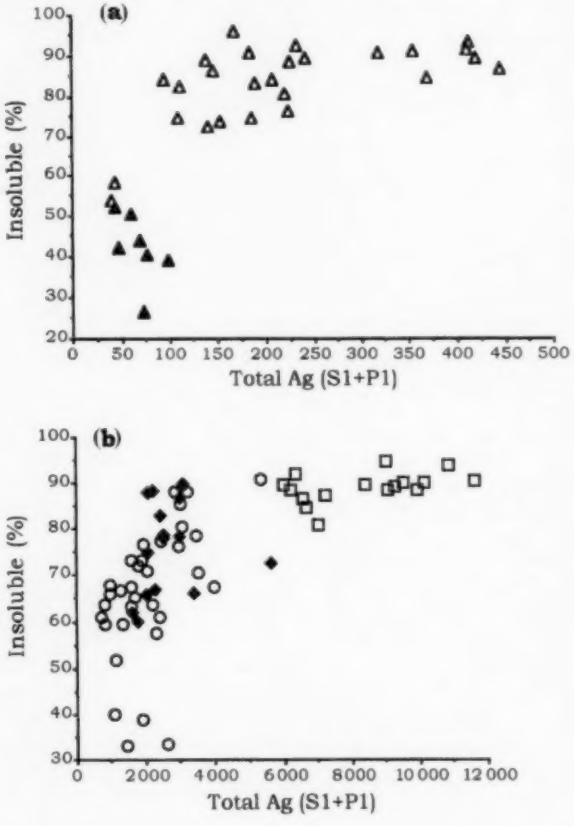


Fig. 2. Relationship between insoluble and total Ag (ng Ag·g wet weight^{-1}) in the soft tissues of *M. balthica*. Influence of the level of exposure: (*a*) controls (△, Loire; ▲, Somme); (*b*) *M. balthica* exposed to 10 (○), 50 (◆), 100 (□) ng Ag·mL^{-1}.



has been described previously (Amiard et al. 1987). The equipment used in this study was a Hitachi Z 8200 spectrophotometer. Standard addition analysis was performed in an isomedium. The analytical methods were validated by intercalibrations organized by IAEA (Coquery and Horvat 1996).

For total Hg, the acid solution prepared previously was completely mineralized using a sulfopermanganate mixture (saturated solution of $KMnO_4$ in H_2SO_4, 3%) at 90°C for 15 min. The excess oxidant was then destroyed by the addition of hydroxylamine. Mercury was then analyzed by cold-vapor atomic absorption spectrophotometry (Laboratory Data Control UV monitor), the reducing agent used to transform the Hg ion to metallic Hg being tin chloride (20% in 2 N HCl). This is the method recommended by Boiteau and Pineau (1988) following an exhaustive review of the available procedures and it was tested in internal quality controls (standard reference materials from the National Research Council of Canada, TORT-1, DORM-1, DOLT-1).

Total metal concentrations in whole soft tissue were recalculated from quantities of trace elements in S1 and P1 fractions determined previously.

Statistical analysis

Differences between groups of bivalves according to conditions of exposure or natural factors were evaluated by analysis of variance (ANOVA) and post hoc comparisons assessed by the multiple range test of Scheffé. These tests, as well as *t* tests, were carried out using a standard statistical package (StatView SE + Graphics).

Results

Bioaccumulation of metals

The relationships between concentrations of S1 or insoluble P1 metals and total metals (S1 + P1) in the soft tissues were examined using all experimental groups (Ag: n = 113; Cd: n = 70; Hg: n = 54).

Silver bioaccumulation

Silver concentrations in whole soft tissues increased with exposure. Positive correlations between Ag concentrations in the S1 or insoluble P1 fraction versus total Ag (S1 + P1) were present, but the slope of the regression curves was considerably higher for P1 than for S1 (Fig. 1). In controls, when the total concentration was higher (in specimens from the Loire estuary compared with those of the Bay of Somme), the percentage of insoluble Ag (P1 × 100/S1 + P1) increased (Fig. 2*a*). When the experimental exposure to Ag was increased, the supplementary Ag accumulated was mainly incorporated into the P1 fraction (Fig. 2*b*).

Cadmium bioaccumulation

Cadmium accumulation in *M. balthica* exposed to 10 ng Cd·mL^{-1} was significantly higher in both fractions in comparison with controls. When Cd concentrations increased in whole soft tissues, the extra accumulated metal was incorporated into both the S1 and P1 fractions, the slope being slightly higher for cytosolic Cd (Fig. 3).

Mercury bioaccumulation

The relationships between concentrations of total Hg and soluble or insoluble Hg in control and exposed bivalves are depicted in Fig. 4*a*. An enlargement is shown for the controls (Fig. 4*b*). Under both experimental conditions, increasing concentrations of total Hg were due to accumulation in both storage forms, the slope being higher in the case of soluble Hg.

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Fig. 3. Relationship between soluble (S1, ○) or insoluble (P1, ▲) Cd and total Cd (S1 + P1) in the soft tissues of *M. balthica* (ng Cd·g wet weight^{-1}) exposed to 10 ng Cd·mL^{-1} or controls.

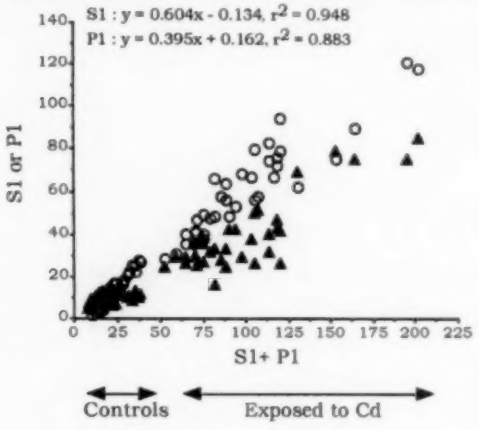
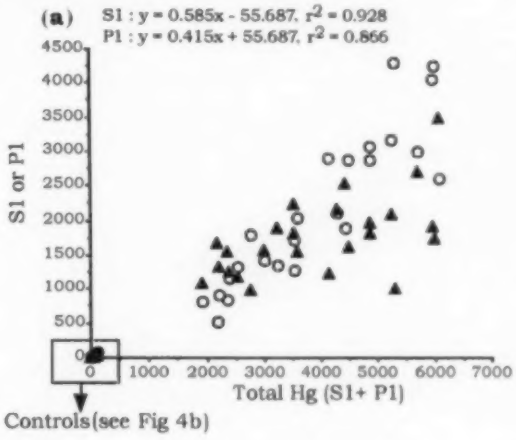
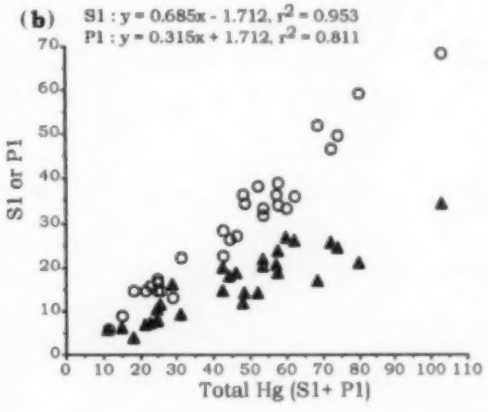


Fig. 4. Relationship between soluble (S1, ○) or insoluble (P1, ▲) Hg and total Hg (S1 + P1) in the soft tissues of *M. balthica* (ng Hg·g wet weight^{-1}) originating from the Loire and Somme estuaries. (*a*) Whole specimens; (*b*) controls only.





MTLP concentrations

Influence of geographical origin

Bivalves originating from both the Loire and Somme estuaries were studied in January and April (Table 1). Since it has been shown previously (Amiard-Triquet et al. 1998) that there is an influence of weight, the comparison was limited to specimens of "medium" size, which were present at both sites. However, in January and April, the condition index was always higher in specimens from the Bay of Somme ($p = 0.0001$), reaching an average 59 and 72 mg·cm^{-3} in January and April, respectively (39 and 60 mg·cm^{-3} in the Loire estuary). The influence of the sampling site on MTLP concentration was examined by comparing the data obtained in bivalves that had been exposed to the same laboratory conditions (bivalves from both the Loire and Somme estuaries exposed in April to 50 and 100 ng Ag·mL^{-1} or 100 ng Hg·mL^{-1}) as well as controls in January and April (Table 2). For each month, two-way ANOVA did not show any influence of the sampling site (January: $n = 15$, $p = 0.1270$; April: $n = 61$, $p = 0.7552$).

Influence of season

In January the field situation is characterized by a low level of available food, whereas in April, bivalves begin to restore their energetic reserves as shown by an increase in condition index (59–72 mg·cm^{-3} in the Bay of Somme and 39–60 mg·cm^{-3} in the Loire estuary). In October (Loire only) the condition index remained statistically unchanged (66 mg·cm^{-3}). The seasonal change in condition index between January and April was more important in the Loire estuary ($p = 0.0001$) than in the Bay of Somme ($p = 0.0628$).

As mentioned above, it is necessary to avoid any potential influence of weight. Consequently, we selected for statistical analysis only individuals in the same weight range (indicated by arrows in Fig. 5).

A first comparison may be carried out between MTLP concentrations of samples collected in January and April. Considering that any influence of geographical origin was shown to be negligible (see above), controls originating from both the Loire and Somme estuaries, as well as clams from the Loire exposed to 100 ng Hg·mL^{-1}, were included in the analysis. MTLP concentrations were significantly higher ($p = 0.0002$) in April than in January (Fig. 5*a*).

Between April and October, the influence of season could be examined only in the population from the Loire estuary (Table 1). No significant differences were noted between the MTLP concentrations of laboratory-exposed bivalves (10 ng Ag·mL^{-1}: $p = 0.2896$; 10 ng Cd·mL^{-1}: $p = 0.3243$; not shown) collected in April and October, respectively, whereas in the controls, MTLP levels were significantly higher in the April than in the October samples ($p = 0.002$) (Fig. 5*b*).

Influence of contamination factors

According to the hypothesis that MTLP is a major sink for bioaccumulated metals, it might be considered preferable to examine relationships between concentrations of MTLP and metal concentrations in the supernatant S2 obtained after heat denaturation of the cytosol (containing heat-stable

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Table 2. MTLP in the soft tissues of *M. balthica* sampled in a clean (Somme) or a contaminated site (Loire).

Sampling period	Experimental conditions	Mean (SD) MTLP concentration ($mg \cdot g^{-1}$) Somme	Loire
January	Control	0.55 (0.07)	0.48 (0.12)
April	Control	0.70 (0.12)	0.70 (0.15)
	50 µg $Ag \cdot L^{-1}$	0.59 (0.09)	0.57 (0.09)
	100 µg $Ag \cdot L^{-1}$	0.67 (0.12)	0.73 (0.09)
	100 µg $Hg \cdot L^{-1}$	0.56 (0.10)	0.61 (0.16)

compounds including MTLP). However, the exact fate of metals during heating is a matter of debate, so we have in fact considered the relationship between MTLP levels and metal levels in the cytosol (S1), as depicted in Fig. 6.

As concluded above, any influence of geographical origin can be ignored. It has also been shown that the influence of season is also negligible in bivalves exposed experimentally to Ag and Cd whereas it must be taken into account when controls are considered. Thus, we have considered here the case of Ag in bivalves originating from both the Loire estuary and the Bay of Somme in April. Only the groups of bivalves showing an intermediate mean weight (medium, Table 1) were selected among clams originating from the Loire estuary (Fig. 6*a*). In October, all the available samples originated from the Loire estuary, but they differed according to the mean weight of their soft tissues (Fig. 6*b*). Experimental Cd exposures were carried out only with specimens from the Loire estuary (Table 1) with three weight-classes in April (Fig. 6*c*) and two in October (Fig. 6*d*). Experimental exposures to Hg were carried out in April only with specimens from both sites (Fig. 6*e*) showing soft tissue weights in the same range (medium, Table 1).

In all the experimental conditions, a positive correlation was observed between MTLP and soluble accumulated metal concentrations, and generally, these relationships were highly significant (Fig. 6). The highest slope was obtained when clams were exposed to Cd.

Influence of weight

The relationship between soft tissue weight and MTLP concentration was studied considering all the groups that did not exhibit MTLP changes in response to one or more of the several factors examined above. On the whole, 74 values fit this condition. A significant negative correlation was shown between the two factors studied (Fig. 7).

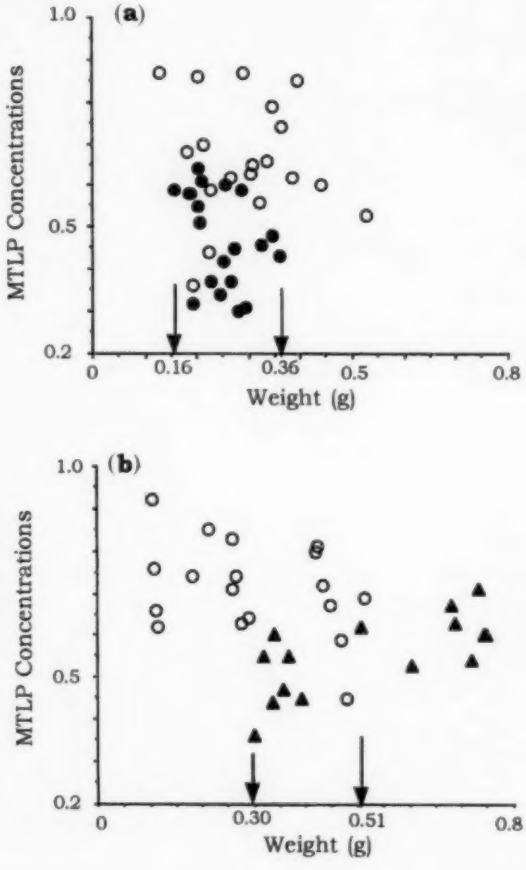
Relative importance of factors influencing MTLP concentrations

Figure 8 shows the ratios between maximum and minimum concentrations of MTLP for each factor for which an influence was shown. It is clear that exposure to metals did not induce changes greater than those observed in relation to changes in natural factors, namely season and weight.

Discussion

Tolerance can result from the ability of organisms to limit the uptake of contaminants. Uptake rates have been compared in *M. balthica* from the Loire estuary and the Bay of Somme exposed to Ag (80 ng stable $Ag \cdot mL^{-1}$ + ^{110m}Ag) or Hg (100 ng stable $Hg \cdot mL^{-1}$ + ^{203}Hg) by Boisson et al. (1998). Lower uptake rates were obtained for Hg (during the initial uptake phase only) and for Ag in clams from the polluted estuary, suggesting that reduced uptake might be an adaptative trait for survival in contaminated areas. However, clams that survived Ag and Hg exposure at LT50 did not protect themselves against metal toxicity by accumulating significantly smaller amounts of these metals than clams that failed to survive the metal stress. Since heavy metal tolerance in *M. balthica* cannot therefore be attributed to lower uptake, attention in the present study was focussed on the physicochemical forms of storage of accumulated metals, particularly MTLPs which are known to intervene in metal detoxification.

Fig. 5. Seasonal changes of MTLP concentrations ($mg \cdot g^{-1}$) in the soft tissues of *M. balthica*. (*a*) Specimens originating from the Loire estuary and the Bay of Somme in January (●) and April (○); (*b*) controls from the Loire estuary in April (○) and October (▲).



Bioaccumulation of metals

When *M. balthica* were experimentally exposed to Ag, Cd, or Hg, incorporation of metals into the soft tissues was observed in all cases, but the distributions of the accumulated metals were different. Silver was predominantly accu-

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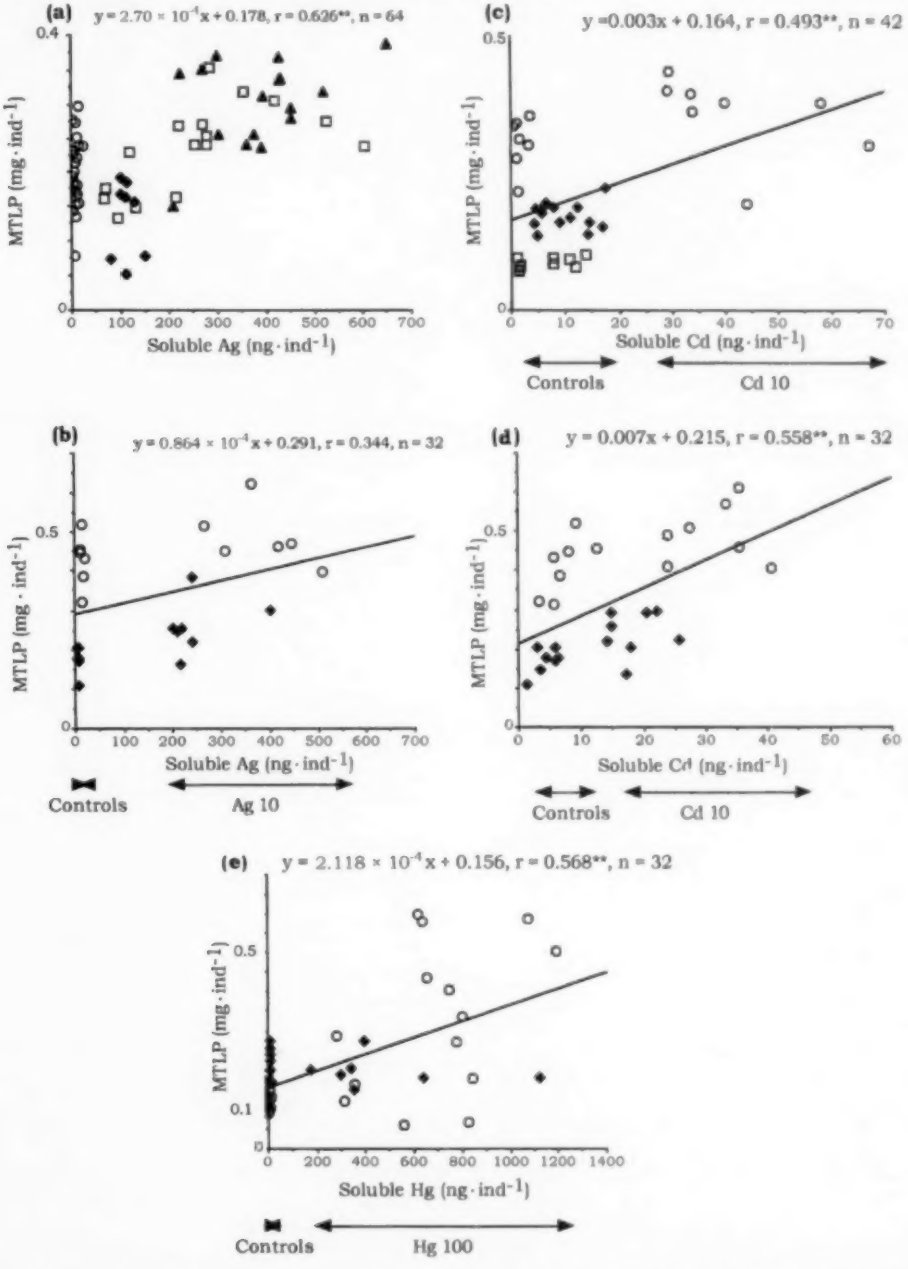
Fig. 6. Relationship between MTLP (mg·individual^{-1}) and metal levels (ng·individual^{-1}) in the soft tissues of *M. balthica*. **Correlation significant at the 99% level. (*a*) Silver in clams sampled in April in the Loire estuary (medium class) and the Bay of Somme: controls (○) and exposed to 10 (◆), 50 (□), or 100 ng·mL^{-1} (▲). (*b*) Silver in clams sampled in October in the Loire estuary, exposed (10 ng·mL^{-1}) or not: ○, large specimens; ◆, medium specimens. (*c*) Cadmium in clams sampled in April in the Loire estuary, exposed (10 ng·mL^{-1}) or not: ○, large specimens; ◆, medium specimens; □, small specimens. (*d*) Cadmium in clams sampled in October in the Loire estuary, exposed (10 ng·mL^{-1}) or not: ○, large specimens; ◆, medium specimens. (*e*) Mercury in clams sampled in April in the Loire estuary (○) and the Bay of Somme (◆), exposed (100 ng·mL^{-1}) or not.


(a)
y = 2.70 × 10⁻⁴x + 0.178, r = 0.626**, n = 64
MTLP (mg · ind⁻¹)
Soluble Ag (ng · ind⁻¹)
(c)
y =0.003x + 0.164, r = 0.493**, n = 42
MTLP (mg · ind⁻¹)
Soluble Cd (ng · ind⁻¹)
Controls
Cd 10
(b)
y = 0.864 × 10⁻⁴x + 0.291, r = 0.344, n = 32
MTLP (mg · ind⁻¹)
Soluble Ag (ng · ind⁻¹)
Controls
Ag 10
(d)
y = 0.007x + 0.215, r = 0.558**, n = 32
MTLP (mg · ind⁻¹)
Soluble Cd (ng · ind⁻¹)
Controls
Cd 10
(e)
y = 2.118 × 10⁻⁴x + 0.156, r = 0.568**, n = 32
MTLP (mg · ind⁻¹)
Soluble Hg (ng · ind⁻¹)
Controls
Hg 100

mulated in the insoluble tissue fraction whereas soluble forms were slightly predominant for Cd and Hg. The predominance of cytosolic Cd has been also reported in *M. balthica* by Langston and Zhou (1987) and in other bivalve species, such as *Mytilus galloprovincialis* (Pavicic et al. 1989; Bebianno and Langston 1992), *Mizuhopecten yessoensis* (Evtushenko et al. 1990), and *C. gigas* (Mouneyrac et al. 1998). In *Crassostrea virginica*, however,

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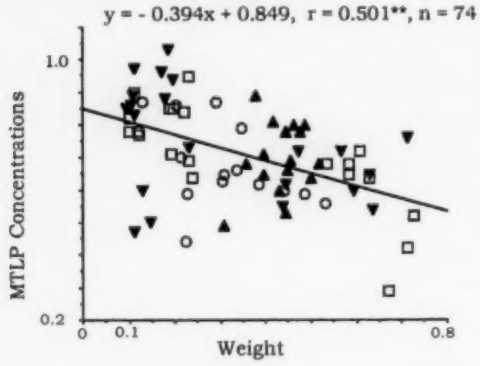
Fig. 7. Relationship between MTLP concentration ($mg \cdot g^{-1}$) and wet weight (g) in the soft tissues of *M. balthica* (see the text for the selection of experimental groups): controls (○) and exposed to 10 (▼) or 100 ng $Ag \cdot mL^{-1}$ (▲) or 10 ng $Cd \cdot mL^{-1}$ (□). **Correlation significant at the 99% level.



the relative importance of cytosolic Cd was highly variable (29–66%) according to site or season (Roesijadi 1994). In *Ruditapes decussatus*, Cd was mainly accumulated in the cytosol, associated with soluble proteins after 48 h of exposure; in contrast, after 7 days of exposure, the percentage of Cd found in the cytosol decreased, suggesting a redistribution of the soluble Cd to the particulate fraction (Romeo and Gnassia-Barelli 1995). In the case of Ag distribution, the present study is in agreement with data reported for other bivalve species (Berthet et al. 1992). In mussels exposed to Hg, the tissue distribution of this element in the gills showed time-dependent changes: the percentage of soluble Hg increased from 25% after 1 day to 91% after 21 days and then decreased again to 58% after 28 days (Roesijadi et al. 1982). This pattern is clearly different from the observations reported in the present study for whole soft tissues of *M. balthica*, but responses are known to be tissue specific and species specific (Langston et al. 1998).

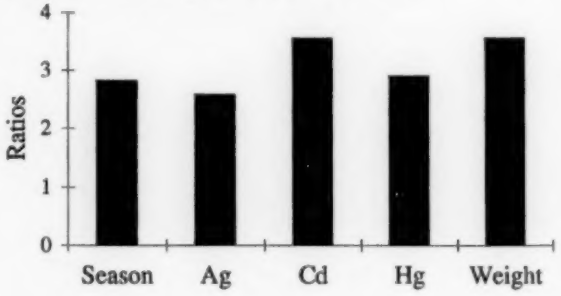
Subcellular components of metal metabolism

The subcellular components of metal metabolism have been recently reviewed (Langston et al. 1998) and include, besides MTs, non-thionein proteins and other cytosolic metal complexes whereas a variety of lysosomes and granules contribute to the insoluble fraction.

In *M. balthica* from the Loire estuary and the Bay of Somme, the present study has revealed the existence of a compound showing some characteristics of MTs: cytosolic, heat stable, detectable by differential pulse polarography through the presence of noticeable levels of S. The presence of this metalloprotein is in agreement with data obtained in populations collected far from the sites investigated in the present study (Arctic estuaries studied by Amiard-Triquet et al. (1998) and the Pacific American coast studied by Bray et al. (1983) and Johansson et al. (1986)) but differs from observations for specimens originating from the nearer south coast of England (Langston and Zhou 1987).

Usually, induction of MT by a low level of toxicants has been interpreted as a process offering resistance to metal exposure in different species, particularly aquatic invertebrates

Fig. 8. Variability of MTLP concentrations (expressed by using ratios between maximum and minimum concentrations) in response to natural factors or exposure to metals.



(Roesijadi et al. 1982). Chronic exposure to metals in the Loire estuary suggested the hypothesis that the clams could have acquired tolerance as a result of elevated levels of detoxication proteins, namely MT. In the present study, no differences have in fact been shown between MTLP levels of clams from the contaminated (Loire estuary) and clean (Bay of Somme) sites. In *R. decussatus*, Bebianno et al. (1993) have shown an involvement of MTLP in the sequestration of excess Cd but have underlined the low level of de novo synthesis of MT at sublethal levels of Cd. Moreover, it is well known that, in contrast with mammals or fish, in invertebrates, MTs frequently do not constitute the major intracellular sink for metals in target tissues (Stegeman et al. 1992).

In *M. balthica* exposed to Ag at a polluted site, Bray et al. (1983) have found that the metal was predominantly bound to proteins with a molecular weight above 100 kDa, with merely 10% of the pollutant bound to a MTLP. In *C. gigas*, Martoja et al. (1988) have reported that soluble Ag was bound to proteinaceous compounds, the characteristics of which were clearly different from those of MTLP. Insoluble Ag, which seems to be the predominant form of storage in various bivalves, is present in several species as silver sulphide, a very stable component, the toxicity of which is therefore limited (Martoja et al. 1988; Berthet et al. 1992).

In *C. virginica* experimentally exposed to 10 or 200 ng $Cd \cdot mL^{-1}$, a major proportion of the metal was bound to high molecular weight compounds (Roesijadi and Klerks 1989). In the case of the lower Cd load, the MT content was equivalent to the "background" physiological content. In the case of the higher Cd load, these authors suggested that the metal binds to other ligands if the induced sites are saturated. Studies carried out by Carpène and George (1981) and Nolan and Duke (1983) suggested that the metal ions initially bind to high molecular weight proteins which could act as intracellular carrier molecules. Once bound to these vector molecules, the metal could then use various pathways including heat-stable MTLPs and other compounds. Similar findings have been reported by Evtushenko et al. (1986) who studied a pectinid (*M. yessoensis*) exposed to an experimental Cd contamination (0.5 $mg \cdot L^{-1}$) for 60 days. They found that the majority of the metal was bound to high molecular weight proteins and that a portion of the metal starts binding to MTLPs only after a prolonged exposure (beyond 60 days). Thus, with a view to understanding the mecha-

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nisms responsible for acquired tolerance, it is necessary to examine all physicochemical forms of storage. Moreover, the transfer of bioaccumulated metals in the food web is greatly influenced according to the detoxification processes involved in tolerance, metals bound to MTLPs being much more available to predators than metals insolubilized as silver sulphide (Martoja et al. 1988; Berthet et al. 1992) or in "granules" (Wallace et al. 1998 and literature cited therein).

Natural factors influencing MTLP levels

The weight of whole soft tissues appears to be an important factor affecting MTLP levels in different populations of *M. balthica* (Amiard-Triquet et al. 1998; present study). The inverse relationship observed between the lowest weights and highest MTLP concentrations has been also reported for metals in bivalves (e.g., Amiard et al. 1994; Mouneyrac et al. 1998) and discussed in depth (National Research Council 1980). These results may be explained at least partly by biological dilution in clams of the most elevated weights (Amiard et al. 1994). In order to eliminate the effect of weight, we examined the relationship between metal and MTLP levels, taking into account the quantity of each constituent in whole soft tissues rather than concentrations.

The influence of season was also been investigated in specimens of similar size. Little influence was noted in controls, and no effect was observed in laboratory-exposed animals. In baltic clams from the western Scheldt estuary, Bordin et al. (1997) have demonstrated an effect of season on MTLP concentrations (higher in winter, lower in summer), a fact that they attributed to body weight fluctuations. Patterns of metal binding to MTs were also significantly affected by date in *C. virginica* (Roesijadi 1994).

With a view to the use of MT as a biomarker, the significant relationship between metal and MTLP levels is satisfying, but the influence of natural factors can interfere in any interpretation of MT data, a situation that was encountered earlier when the feasibility of the International Mussel Watch was discussed (National Research Council 1980). In order to minimize the influence of weight, it is recommended that specimens of comparable size be selected during sampling operations. In the case of seasonal and interannual variations, deseasonalizing methods and analysis of time series may be used (Cossa 1989).

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References

Amiard, J.C., and Cosson, R.P. 1997. Les métallothionéines. *In* Biomarqueurs en écotoxicologie. Aspects fondamentaux. *Edited by* L. Lagadic, T. Caquet, J.C. Amiard, and F. Ramade. Masson, Paris, France. pp. 53–63.

Amiard, J.C., Pineau, A., Boiteau, H.L., Métayer, C., and Amiard-Triquet, C. 1987. Application of atomic absorption spectrophotometry using Zeeman effect to the determination of eight trace elements (Ag, Cd, Cr, Cu, Mn, Ni, Pb and Se) in biological materials. Water Res. **21**: 693–697.

Amiard, J.C., Métayer, C., Baud, J.P., and Ribeyre, F. 1994. Influence of some ecological and biological factors on metal bioaccumulation in young oysters (*Crassostrea gigas* Thunberg) during their spat rearing. Water Res. **28**: 219–231.

Amiard-Triquet, C., Rainglet, F., Larroux, C., Regoli, F., and Hummel, H. 1998. Metallothioneins in arctic bivalves. Ecotoxicol. Environ. Saf. **41**: 96–102.

Bebianno, M.J., and Langston, W.J. 1992. Cadmium induction of metallothionein synthesis in *Mytilus galloprovincialis*. Comp. Biochem. Physiol. C, Comp. Pharmacol. **103**: 79–85.

Bebianno, M.J., Nott, J.A., and Langston, W.J. 1993. Cadmium metabolism in the clam *Ruditapes decussata*: the role of metallothioneins. Aquat. Toxicol. **27**: 315–334.

Berthet, B., Amiard, J.C., Amiard-Triquet, C., Martoja, M., and Jeantet, A.Y. 1992. Bioaccumulation, toxicity and physicochemical speciation of silver in bivalve molluscs: ecotoxicological and health consequences. Sci. Total Environ. **125**: 97–122.

Bliss, C.I. 1938. The determination of the dosage mortality curve from small numbers. Q. J. Pharmacol. **2**: 192–216.

Boisson, F., Hartl, M.G.J., Fowler, S.W., and Amiard-Triquet, C. 1998. Influence of chronic exposure to silver and mercury in the field on the bioaccumulation potential of the bivalve *Macoma balthica*. Mar. Environ. Res. **45**: 325–340.

Boiteau, H.L., and Pineau, A. 1988. Mercury. *In* Quantitative trace analysis of biological materials. *Edited by* A. McKenzie. Elsevier, Amsterdam, The Netherlands. pp. 553–560.

Bordin, G., Raposo, F.C., McCourt, J., and Rodriguez, A.R. 1994. Occurrence of metallothionein-like metal binding proteins in the marine bivalve *Macoma balthica*. C. R. Acad. Sci. Paris, **317**: 1057–1064.

Bordin, G., McCourt, J., Cordeiro-Raposo, F., and Rodriguez, A.R. 1997. Metallothionein-like metalloproteins in the Baltic clam *Macoma balthica*: seasonal variations and induction upon metal exposure. Mar. Biol. **129**: 453–463.

Bray, J.T., Webb, L.A., and Reilly, F.J., Jr 1983. Multielement analysis of metal-binding proteins in cytosolic fractions. Sci. Total Environ. **28**: 367–374.

Carpene, E., and George, S.J. 1981. Absorption of Cd by gills of *Mytilus edulis* (L). Mol. Physiol. **1**: 23–24.

Coquery, M., and Horvat, M. 1996. The analytical performance study for the MEDPOL area: determination of trace-elements in marine sediment SD-MEDPOL-1/TM and fish homogenate MA-MEDPOL-1/TM. Report IAEA, Intern. Agency Energy Atomic, Monaco.

Cossa, D. 1989. Sampling strategies and normalization for optimizing the use of *Mytilus* spp. as quantitative indicators of metal contamination. Oceanol. Acta, **12**: 417–432.

Evtushenko, Z.S., Belcheva, N.N., and Lukyanova, O.N. 1986. Cadmium accumulation in organs of the scallop *Mizuhopecten yessoensis*. II. Subcellular distribution of metals and metal-binding proteins. Comp. Biochem. Physiol. C, Comp. Pharmacol. **83**: 377–383.

Evtushenko, Z.S., Lukyanova, O.N., and Belcheva, N.N. 1990. Cadmium bioaccumulation in organs of the scallop *Mizuhopecten yessoensis*. Mar. Biol. **104**: 247–250.

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George, S.G., and Olsson, P.E. 1994. Metallothioneins as indicators of trace metal pollution. *In* Biomonitoring of coastal waters and estuaries. *Edited by* K.J.M. Kramer. CRC Press, Boca Raton, Fla. pp. 151–178.

Johansson, C., Cain, D.J., and Luoma, S.N. 1986. Variability in the fractionation of Cu, Ag and Zn among cytosolic proteins in the bivalve *Macoma balthica*. Mar. Ecol. Prog. Ser. **28**: 87–97.

Klerks, P.L., and Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut. **45**: 173–205.

Langston, W.J., and Zhou, M. 1987. Cadmium accumulation, distribution and elimination in the bivalve *Macoma balthica*: neither metallothionein nor metallothionein-like proteins are involved. Mar. Environ. Res. **21**: 225–237.

Langston, W.J., Bebianno, M.J., and Burt, G.R. 1998. Metal handling strategies in molluscs. *In* Metal metabolism in aquatic environments. *Edited by* W.J. Langston and M.J. Bebianno. Chapman and Hall, London, U.K. pp. 219–283.

Martoja, R., Ballan-Dufrançais, C., Jeantet, A.Y., Gouzerh, P., Amiard, J.C., Amiard-Triquet, C., Berthet, B., and Baud, J.P. 1988. Effets chimiques et cytologiques de la contamination expérimentale de l'huître *Crassostrea gigas* Thunberg par l'argent administré sous forme dissoute ou par voie alimentaire. Can. J. Fish. Aquat. Sci. **45**: 1827–1841.

Mouneyrac, C., Amiard, J.C., and Amiard-Triquet, C. 1998. Effects of natural factors (salinity and body weight) on cadmium, copper, zinc and metallothionein-like protein levels in resident populations of oysters *Crassostrea gigas* from a polluted estuary. Mar. Ecol. Prog. Ser. **162**: 125–135.

National Research Council. 1980. The International Mussel Watch. Report of a workshop sponsored by the environmental studies board. Natural Resources Commission, Washington, D.C.

Nolan, C.V., and Duke, E.J. 1983. Cadmium accumulation and toxicity in *Mytilus edulis*: involvement of metallothioneins and heavy-molecular weight protein. Aquat. Toxicol. **4**: 153–163.

Pavicic, J., Skreblin, M., Kregar, I., Tuzzek-Znidaric, M., and Stegnar, P. 1989. Determination of Cd-binding proteins similar to metallothioneins in the digestive gland of *Mytilus galloprovincialis* in relation to the preliminary treatment of the sample. Period. Biol. **911**: 213–224.

Réseau National d'Observation. 1995. Les contaminants dans la matière vivante. *In* Surveillance du milieu marin. *Edited by* Min. Environ., Paris, and IFREMER, Nantes. pp. 9–24.

Rigaa, A., Ajjou, M., and Sellos, D. 1998. Partial cDNA sequence of a metallothionein-like protein with new features from the bivalve *Macoma balthica*. J. Mar. Biotechnol. **6**: 83–85.

Roesijadi, G. 1994. Behavior of metallothionein-bound metals in a natural population of an estuarine mollusc. Mar. Environ. Res. **38**: 147–168.

Roesijadi, G., and Klerks, P.L. 1989. Kinetic analysis of cadmium binding to metallothionein and other intracellular ligands in oyster gills. J. Exp. Zool. **251**: 1–12.

Roesijadi, G., Drum, A.S., Thomas, J.M., and Fellingham, G.W. 1982. Enhanced mercury tolerance in marine mussels and relationship to low molecular weight mercury-binding proteins. Mar. Pollut. Bull. **13**: 250–253.

Romeo, M., and Gnassia-Barelli, M. 1995. Metal distribution in different tissues and in subcellular fractions of the Mediterranean clam *Ruditapes decussatus* treated with cadmium, copper or zinc. Comp. Biochem. Physiol. C, Comp. Pharmacol. **111**: 457–463.

Stegeman, J.J., Brouwer, M., Di Giulio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M., and Van Veld, P.A. 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. *In* Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. *Edited by* R.J. Huggett, R.A. Kimerle, P.M. Mehrle, and H.L. Bergman. Lewis Publishers, Chelsea, Mich. pp. 235–335.

Thompson, J.A.J., and Cosson, R.P. 1984. An improved electrochemical method for the quantification of metallothionein in marine organisms. Mar. Environ. Res. **11**: 137–152.

Wallace, W.G., Lopez, G.R., and Levinton, J.S. 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. **172**: 225–237.

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Bayesian state–space modeling of age-structured data: fitting a model is just the beginning

Russell B. Millar and Renate Meyer

Abstract: Explicit modeling of process variability in the dynamics of fisheries is motivated by a desire to incorporate more realism into stock assessment models, and much recent research effort has been devoted to the computational features of fitting state–space models for this purpose. Here, we extend the Bayesian application of nonlinear state–space modeling to sequential population analysis of age-structured data using a model formulation that allows for unreported catches and incidental fishing mortality. It is shown that, once a familiarity with the general-purpose Bayesian software BUGS is acquired, implementing a state–space model is a relatively simple task. Indeed, this application requires just 18 lines of code in its entirety and does not require the programmer to know the formulae for any prior density functions or likelihoods. Consequently, we suggest that this methodology may permit the implementation phase of nonlinear state–space modeling to be relegated, thereby allowing more effort to be devoted to the challenging issues of model checking, selection/averaging, sensitivity, and prior specification.

Résumé : La modélisation explicite de la variabilité des processus en dynamique des pêches découle de l'intention de rendre plus réalistes les modèles d'évaluation des stocks, et beaucoup de recherches ont été consacrées récemment aux caractéristiques computationnelles de l'ajustement des modèles état–espace. Dans le présent cas, nous étendons l'application bayésienne de la modélisation non-linéaire état–espace à l'analyse séquentielle de population de données structurées par âges en faisant appel à une formulation du modèle qui permet de tenir compte des captures non signalées et de la mortalité par pêche accidentelle. Il est montré que l'utilisation d'un modèle état–espace s'avère relativement simple une fois que l'on connaît assez bien le logiciel bayésien général BUGS. Une telle application n'exige que 18 lignes de codes au total et le programmeur n'a pas à connaître les formules des fonctions de densité ou de ressemblance antérieures. Nous croyons donc que cette méthode pourrait permettre de délaisser l'étape de mise en oeuvre de la modélisation non-linéaire état–espace et ainsi de consacrer plus d'efforts aux questions plus intéressantes de la vérification, du choix/de la mise en moyenne, de la sensibilité et de la définition antérieure des modèles.

[Traduit par la Rédaction]

Introduction

A model provides a conceptual framework for learning about reality. It is necessarily a simplification, but must retain the ability to capture the features that are required to address questions of interest. In fisheries stock assessment the modeling considerations are manifold. In addition to the question of appropriate functional specification (e.g., Fox or Schaefer surplus production, depensation at low stock size, hyperstability of catch-per-unit-effort data), other vital issues include the ability of the model to cope with the frequent shortcomings of the data (e.g., misreported catches, ageing errors, correlation) and the effects of process randomness (e.g., variability in population dynamics).

Much recent effort has been invested in using state–space methodology to incorporate process randomness in stock assessment models (e.g., Sullivan 1992; Pella 1993; Gudmundsson 1994, 1995; Schnute 1994; Freeman and Kirkwood 1995; Kimura et al. 1996; Reed and Simons 1996). State–space models can also incorporate variability in key population parameters (e.g., carrying capacity or natural mortality) by allowing the parameter to follow a first-order autoregressive process over time. In contrast, models without process error imply deterministic biomass trajectories and time-invariant parameters. Under these assumptions, a surplus production model (e.g., Schaefer 1954; Hilborn and Walters 1992) predicts the exact biomass of the stock into the infinite future as a deterministic function of the current biomass and harvesting policy.

Most of the existing work on state–space modeling of fisheries dynamics has used classical likelihood-based statistical theory, as distinct from the Bayesian approach used here. Calculation of the likelihood function requires high-dimensional integration because it is necessary to integrate over the unobserved process errors (see Schnute 1994). A tractable integral is obtained when the model is linear and has normally distributed errors, whence the fit can be achieved using the Kalman filter (see Meinhold and Singpurwalla 1983). However, linearity and normality greatly restrict the realism and general applicability of the state–space methodology. In the case of nonlinear state–space models, the computational intractability results in the use of approximate fits such as that obtained using linear approximation (the extended Kalman filter). Despite considerable interest in state–space models, and indications that

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they *do* have superior performance compared with deterministic models (Anonymous 1995), they are not widely used because of these complications.

Some fisheries state–space models have been fitted using penalized likelihood (e.g., Ludwig et al. 1988; Schnute 1994; Richards and Schnute 1998) as an alternative to standard likelihood. This approach treats process errors as fixed parameters to be estimated, and hence, fitting of the model reduces to a conceptually simple maximization problem because the likelihood for the observables is an explicit function of the specified values of the model parameters and process errors. This methodology has the advantage of being very generally applicable, but the disadvantage of undesirable properties under common circumstances. For example, when using penalized likelihood to fit generalized linear mixed models, the estimates of fixed effects are not consistent when there are limited data per random effect (which is typically the case), for which asymptotic bias correction formulae have been provided by Lin and Breslow (1996).

Tractability concerns are not as relevant to fully Bayesian state–space models (e.g., Carlin et al. 1992) because the Markov chain Monte Carlo (MCMC) techniques used for sampling from the joint posterior are very general in their application. These are numerically intensive techniques that do not rely on algebraic simplifications being obtained from assumptions such as linearity and normality. Millar and Meyer (2000) and Meyer and Millar (1999*a*) implemented surplus production and delay-difference state–space models, respectively, and used C++ to perform the MCMC sampling. Recently, the use of C++ has become unnecessary due to enhancements to the BUGS (Bayesian inference using Gibbs sampling) software package (Gilks et al. 1994; Spiegelhalter et al. 1996). Meyer and Millar (1999*b*) introduced the BUGS software and showed how it provides a convenient implementation of the Bayesian state–space surplus production model of Millar and Meyer (2000).

The use of MCMC does require some care. The joint posterior distribution is the equilibrium distribution of the Markov chain. Hence, it is necessary to check that the Markov chain has been run for sufficiently long to ensure that it is sampling from its equilibrium distribution (this is called "burning in" of the chain). In addition, it must be checked whether the chain is "mixing" well, in the sense of being able to move easily through the possibly high-dimensional space of the posterior distribution. These concerns are briefly examined in the context of the example presented herein. See Meyer and Millar (1999*b*) for more detail.

The purposes of this article are twofold. First, the application of Bayesian state–space models is extended to age-structured data. In its entirety, the implementation requires 18 lines of BUGS code, and at no stage is there a requirement for the modeler to know the details (e.g., formulae) for density functions or likelihoods. A knowledge of modeling at the level of Hilborn and Mangel (1997) is sufficient. Second, we consider the wider consequences of the demonstrated flexibility and expedience of implementation. In particular, we suggest that the process of stock assessment modeling should now focus on the more important issues of model checking and selection (or averaging). Of course, in the Bayesian paradigm, there is also the need to consider prior specification.

Age-structured state–space model

The model presented here is developed for an example where the data are indices of numbers-at-age obtained from research vessel surveys. Reported catch-at-age numbers are also utilized, but these are treated as constants and are not modeled as response variables. (In assessments where reliable commercial effort data are available, then treating reported catch as a response may be a sensible modification to the model.) Moreover, the model does not rely on the reported catches being accurate or precise and they are simply regarded as one component of the true overall fishing mortality.

Randomness in the fisheries dynamics is incorporated by dispensing with the concept of a fixed (and typically "known") natural mortality. In fact, here, the notation m^+ will be used to denote *all* mortality in addition to the reported catches. It therefore includes natural mortality, unreported catches, and unaccounted fishing mortality arising from discarding, high-grading, ghost fishing, and injury of fish escaping the gear (e.g., Sangster et al. 1996). (See Chopin et al. (1996) for an in depth discussion of unaccounted mortality.) Unaccounted mortality can be a major problem in some fisheries; for example, Myers et al. (1997) argued that high unaccounted mortality of juveniles was a contributing cause of the collapse of six Canadian populations of Atlantic cod (*Gadus morhua*).

The data and model formulation used here differ substantively from the likelihood-based age-structured model used by Gudmundsson (1994). However, they do have in common the modeling of mortality, and consequently, both are nonlinear models. Gudmundsson (1994) used the extended Kalman filter to obtain an approximate maximum likelihood fit. In the Bayesian implementation, the BUGS software is sufficiently versatile at performing MCMC sampling that the nonlinearity does not present any additional difficulties.

Process equation

If $N_{a,y}$ is the true number of age a fish at the start of year y and $C_{a,y}$ is the catch taken during year y, then the cohort equation is frequently written as

$$(1) \qquad N_{a,y} = N_{a-1,y-1}\exp(-m) - C_{a-1,y-1}\exp(-0.5m)$$

where m is natural mortality. This model is derived from assuming that fishing occurs in the middle of the year and is known to be insensitive to this assumption (Mertz and Myers 1996).

Of course, in reality, the catches are not known exactly, m may vary, and other sources of fishing-related mortality are likely. There are a variety of ways to incorporate variability in eq. 1 (see Discussion). Here, the cohort equation was modified by putting age and year subscripts and a "+" superscript on the quantity m, giving

$$(2) \qquad N_{a,y} = N_{a-1,y-1}\exp(-m^+_{a-1,y-1}) - C_{a-1,y-1}\exp(-0.5m^+_{a-1,y-1}).$$

The biological interpretation of eq. 2 effectively gives $m^+_{a,y}$ the desired definition of all mortality on age a fish in year y beyond that accounted for by the reported catches.

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Observation equation

The published research vessel indices and catches of Myers and Cadigan (1995*b*) are used here. Their table 1A gives relative indices of numbers-at-age, $I_{a,y}$, from autumn research surveys on the 2J–3KL stock of Atlantic cod from 1978 through 1993 and for ages 3–12. Table 1B of Myers and Cadigan (1995*b*) gives the reported catches-at-age, $C_{a,y}$, for age a in calendar year y. Myers and Cadigan (1995*a*, 1995*b*) assumed the indices $I_{a,y}$ to have lognormal distributions. However, table 1A of Myers and Cadigan (1995*b*) contains five indices that, to two decimal places, were rounded down to zero. Here, these five indices were given the value of 0.001 so that they would not violate the assumption of lognormal distribution.

The autumn surveys for 2J–3KL cod take place largely in November. If the numbers-at-age at the time of the survey are denoted $N^I_{a,y}$, then the log of numbers-at-age at the time of the survey is well approximated by

$$\log(N^I_{a,y}) = (\log(N_{a,y}) + 11\log(N_{a+1,y+1}))/12. \quad (3)$$

There are a variety of choices for modeling the distribution of the relative abundance index $I_{a,y}$. The lognormal distribution is generally considered a sensible choice for a relative abundance index, whence

$$I_{a,y} \sim \mathrm{LN}(\log(q_a N^I_{a,y}), \sigma^2) \quad (4)$$

where ~ denotes "distributed as," $\mathrm{LN}(\cdot,\cdot)$ denotes the lognormal distribution (with parameters being the mean and variance of the log of the variable), and the q_as are age-dependent catchabilities, i.e.:

$$\log(I_{a,y}) = \log(q_a N^I_{a,y}) + \epsilon_{a,y} \quad (5)$$

where $\epsilon_{a,y}$ are independent $N(0,\sigma^2)$ distributed error terms. The expected value of $\log(I_{a,y})$ is $\log(q_a N^I_{a,y})$, which (from eq. 3) can be written as

$$\log(q_a N^I_{a,y}) = \log(q_a) + (\log(N_{a,y}) + 11\log(N_{a+1,y+1}))/12\,. \quad (6)$$

Priors

Bayesian implementation of this model requires specification of prior distributions on all unobserved quantities (these are denoted in Appendix A). The specification of some of these priors has parallels with the classical methodology for sequential population analysis where, for example, auxiliary information is used to specify various mortality rates. In particular, virtual population analyses (VPAs) of the 2J–3KL cod stock have traditionally fixed m at 0.2 and regarded this quantity as natural mortality. Recall that here, this quantity is denoted $m^+_{a,y}$ because it is permitted to vary with age (of cohort) and year and denotes all mortality in addition to the reported catch. For example, the distribution of $m^+_{a,y}$ can be made age specific by use of a hierarchical prior of the form

$$m^+_{a,y} \sim \mathrm{LN}(\mu_a, \tau^2),\ \mu_a \sim N(\nu, \sigma^2),\ \tau^2 \sim \mathrm{IG}(\alpha, \beta) \quad (7)$$

where IG denotes the inverse gamma distribution. Setting $\nu = \log(0.2)$ gives each $m^+_{a,y}$ a prior median value of 0.2. A high value of σ^2 would then represent little prior confidence in the presumption that $m^+_{a,y}$s are close to 0.2 and age independent. This particular prior specification does not explicitly model a year effect on the $m^+_{a,y}$, notwithstanding that choosing α and β to give a highly dispersed hyperprior distribution for τ^2 correponds to little prior belief in $m^+_{a,y}$ being constant over time within each age-class.

In a VPA, it is necessary to specify terminal mortalities for age A fish in each year and for all ages in the most recent year. For the model herein the analogy of this is the requirement to place priors on recruitment (in all years) and numbers-at-age in the initial year ($y = 1$). Lack of knowledge about these quantities would be represented by use of a noninformative prior or a diffuse prior.

The wider issue of specifying priors is deferred to the Discussion, where we discuss this aspect of model specification in conjunction with the issues of model selection and checking.

Implementation

In a Bayesian example, "analysis" corresponds to determining the posterior distribution of unknowns of interest. The BUGS software is an MCMC tool that samples numerically from the joint posterior of the unknown quantities (Gilks et al. 1996), subject to the chain being run sufficiently long to ensure burn-in. See Meyer and Millar (1999*b*) for details of the operation of BUGS in the context of a surplus production model.

Programs implemented in BUGS can be expressed in text form (Appendix A) or represented graphically (Fig. 1). For this catch-at-age example, the heart of the program (lines 02 to 12, Appendix A) is simply the requirement to write eqs. 2, 4, and 7 within the permitted syntax of BUGS. (For the program, the age and years have been translated such that $a = 1$ and $y = 1$ are the youngest age and first year for which data are available.) The latter part of the BUGS code (lines 13 to 18) is specifying prior distributions for unobserved quantities. (BUGS does not allow improper priors to be used, and hence, noninformative improper priors are approximated by diffuse proper priors.) Meyer and Millar (1999*a*) showed how the statistical risks associated with various harvest and management scenarios can be assessed by minor addition to the BUGS program.

As an indication of the type of "results" provided by this example, density plots of 9500 samples from the posterior distribution of numbers-at-age at the start of 1983 are displayed in Fig 2. These were obtained from a run of length 100 000 in which a thinning of 10 was used, i.e., only every 10th sample was saved to disk. This is a useful strategy when successive samples are correlated and also keeps the output file down to a reasonable size. Convergence diagnostics were checked using CODA (Best et al. 1995), and burn-in was achieved after discarding the first 500 of the saved samples.

Discussion

The previous section constructs and fits an age-structured model in which the state–space formulation of eq. 2 permits a plausible cohort equation with a biological interpretation. However, it would be inappropriate to use the results from this model for inference about current stock status or for risk management. There are many challenging issues that must first be addressed. A non-exhaustive list follows.

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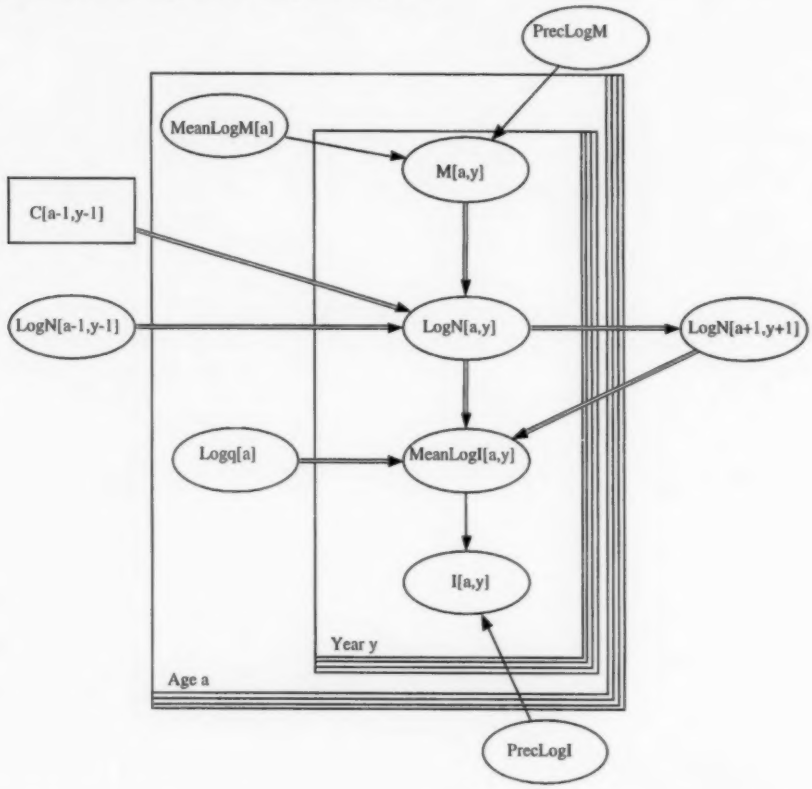
Fig. 1. Directed acylic graph of the model in Appendix A. Logical links are shown using a double arrow and correspond to use of the <- symbol in Appendix A. Stochastic links use a single arrow and correspond to the ~ symbol in Appendix A. Catch-at-age numbers are treated as constants and hence are represented using a rectangular box.



(1) Alternative formulations to eq. 2.
   —For example, northern cod are traditionally analysed using a VPA model that reconstructs the cohorts backwards in time and requires assumptions about terminal fishing mortalities.
   —True catches could be modeled as unobservables and reported catches used as measurements on these unobservables.
(2) Use of additional data.
   —Commerical effort data could be used to model reported catch.
   —Further structure could be imposed on $m^{+}_{a,y}$ by using environmental data or assuming that incidental mortality is related to fishing effort. For example, if E_y is the fishing effort in year y and d_a is a measure of instantaneous age-dependent incidental mortality, then the incidental mortality component of $m^{+}_{a,y}$ could be modeled by adding a term proportional to $d_a E_y$.
(3) Paradigm shifts, such as increased natural mortality, could be modeled by imposing temporal autoregressive structure on $m^{+}_{a,y}$. Similar consideration could be given to putting autoregressive structure on recruitment.
(4) Ageing errors.
(5) Correlated research survey indices, $I_{a,y}$, within years.
(6) Specification of priors.
(7) Sensitivity to priors.

The model fitting considerations in items 1 to 5 are revelant to any age-structured analysis. There are two features of the model fitting process that are particularly relevant to the Bayesian state–space approach presented here: prior specification and flexibility of implementation.

Prior specification

The model formulation (Appendix A) requires prior specification of $m^{+}_{a,y}$, q_a, σ^2 (observation variance), and of the top and left margins of the table of numbers-at-age (i.e., the age structure in the first year and subsequent recruitments). Opponents to Bayesian methodology would argue that this daunting task is sufficient reason to dismiss this Bayesian model as unworkable. However, such an expedient dismissal overlooks the reality that classical VPA requires similar prior knowledge. For example, m is typically assumed to be *constant* and *known*, structure on catchabilities by age, q_a, is imposed, the ratio of error variances is assumed (e.g., Richards and Schnute 1998), and terminal mortalities must be specified or derived.

When prior knowledge is not available, then noninformative priors can often be derived. However, much current work is aimed at obtaining formally derived informative priors using hierarchical analysis (Hilborn and Liermann

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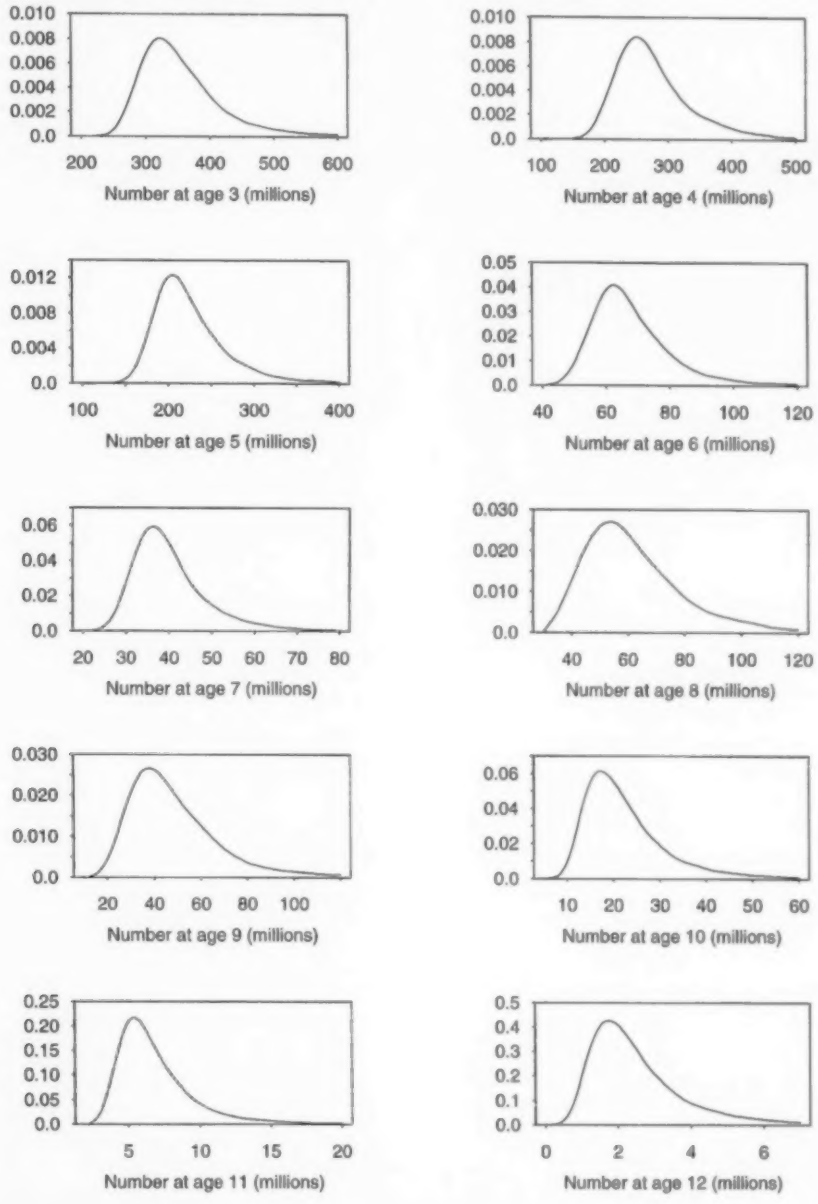
Fig. 2. Posterior distributions for numbers-at-age at the start of 1983, obtained from 100 000 iterations of the Gibbs sampler. Every 10th sample was saved, and the first 500 of the 10 000 saved samples were discarded to allow for burn-in (i.e., convergence of the chain). The plots used the remaining 9500 samples.


Number at age 3 (millions)
Number at age 4 (millions)
Number at age 5 (millions)
Number at age 6 (millions)
Number at age 7 (millions)
Number at age 8 (millions)
Number at age 9 (millions)
Number at age 10 (millions)
Number at age 11 (millions)
Number at age 12 (millions)

1998; Myers et al. 1999). Finally, recommendations for construction of vaguely informative priors can be utilized (e.g., Spiegelhalter et al. 1994; Gelman et al. 1995), along with suitable sensitivity analysis.

Flexibility

BUGS is a numerically intensive software package that implements general Bayesian models using "Metropolis–Hastings within Gibbs sampling" (Gilks 1996). It does not rely on simplifying assumptions (e.g., linearity, normality) for the very reason that it employs a numerical technique for sampling from the joint posterior. In addition, all commonly used (and many other) statistical distributions are recognized by BUGS, and this allows a vast range of models to be implemented in BUGS without the need to specify formulae for densities or likelihoods. With few exceptions, once the model has been expressed (in the form of eqs. 2 and 4, for example), then it can be transcribed into a BUGS program.

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Thus, using BUGS for stock assessment is quite different from using prewritten computer code, spreadsheets, or specific modules, functions, or procedures within statistical software packages, which typically constrain the user to a particular class of model and analysis.

Some of the above alternative models can be implemented with very little change to the BUGS code (Appendix A). For example, Myers and Cadigan (1995*a*) detected a significant within-year correlation in the research survey indices $I_{a,y}$ and modeled this as a normally distributed random effect on the log scale. Appendix B shows that the BUGS code required to implement this alternative differs from the BUGS code of Appendix A by just two lines.

We cannot guarantee that all the model alternatives listed above (or of interest to the reader) can be fitted with ease using BUGS. However, the potential for exploring a vast number of (possibly very complex) models is definitely immense.

Looking ahead

The great flexibility of implementation and the possibilities for alternative prior specification combine to create a bewildering set of competing models. Model checking (e.g., Gelman et al. 1996) and sensitivity analysis will of course be vital tools, and model averaging may be a useful approach (e.g., Raftery 1996).

The issue of prior specification is receiving considerable research attention, both theoretical and applied, within the statistical and fisheries communities (e.g., Sun and Berger 1998; Myers et al. 1999). These efforts will establish guidelines for prior specification, and formally derived priors will become available for certain key population parameters. Nonetheless, there will never be an "absolute" regarding the specification of priors, and many modelers therefore see the need for priors as the Achilles heel of Bayesian methodology. However, it is also the strength of the Bayesian approach — it is the only coherent statistical methodology for updating knowledge using the information contained in data (De Finetti 1974). This property enables the posterior from one analysis to be used as an induced prior in a subsequent analysis, thereby building and exploiting an accumulated base of knowledge.

There is a moderate learning curve to ascend before one can write a complete BUGS program. However, anyone who is comfortable with model formulae and has an intuitive grasp of fundamental statistical concepts should be capable of understanding, using, and modifying BUGS code that has already been written for a particular stock assessment task. For a Bayesian analysis, the required statistical knowledge includes familiarity with the Bayesian paradigm and an appreciation for the numerically intensive methods used to sample from the posterior distribution.

Knowledge, preferably first-hand, of the stock is required for either a Bayesian or a classical model. If the tribulations of model implementation are reduced, then there may be an opportunity to address some of the concerns identified by Rose (1997). In particular, looking beyond the mere fitting of the model, the issues of model selection and prior specification can not be fully answered from the desktop. The modeler will have to go further afield to obtain a greater understanding of the aquatic system for which he or she has the responsibility of modeling.

The example herein is the most sophisticated that we have yet fitted using BUGS and is perhaps at the present limit of practical usability in terms of computational requirements. A 233-MHz laptop generates about 5000 samples per hour from the joint posterior. However, many useful models for stock assessment will be of lesser complexity; for example, the state–space surplus production model used in Meyer and Millar (1999*b*) generated timely results at the rate of 100 samples per second from the joint posterior. We are optimistic that the slow speed of the age-structured model presented herein will be just a transient difficulty. Continual improvements to the BUGS software are enhancing its performance and capabilities, and indeed, it was simply not possible to implement any of these state–space models in the 1997 versions of BUGS. Moreover, the most successful speed-ups are often obtained via minor changes to the code (such as reparameterization), and this is an area that requires further exploration.

References

Anonymous. 1995. Report of the working group on methods of fish stock assessments. ICES Coop. Res. Rep. No. 199.

Best, N.G., Cowles, M.K., and Vines, S.K. 1995. CODA manual version 0.30. MRC Biostatistics Unit, Cambridge, U.K.

Carlin, B.P., Polson, N.G., and Stoffer, D.S. 1992. A Monte Carlo approach to nonnormal and nonlinear state–space modeling. J. Am. Stat. Assoc. **87**: 493–500.

Chopin, F., Inoue, Y., and Arimoto, T. 1996. Development of a catch mortality model. Fish. Res. **25**: 377–382.

De Finetti, B. 1974. Theory of probability; a critical introductory treatment. Vol 1. Wiley, London, U.K. Translated from Italian.

Freeman, S.N., and Kirkwood, G.P. 1995. On a structural time series method for estimating stock biomass and recruitment from catch and effort data. Fish. Res. **22**: 77–98.

Gelman, A., Carlin, J.B., Stern, H.S., and Rubin, D.B. 1995. Bayesian data analysis. Chapman and Hall, London, U.K.

Gelman, A., Meng, X., and Stern, H. 1996. Posterior predictive assessment of model fitness via realized discrepancies (with discussion). Stat. Sinica, **6**: 733–807.

Gilks, W.R. 1996. Full conditional distributions. *In* Markov chain Monte Carlo in practice. *Edited by* W.R. Gilks, S. Richardson, and D.J. Spiegelhalter. Chapman and Hall, London, U.K. pp. 75–88.

Gilks, W.R., Thomas, A., and Spiegelhalter, D.J. 1994. A language and program for complex Bayesian modelling. Statistician, **43**: 169–178.

Gilks, W.R., Richardson, S., and Spiegelhalter, D.J. 1996. Introducing Markov chain Monte Carlo. *In* Markov chain Monte Carlo in practice. *Edited by* W.R. Gilks, S. Richardson, and D.J. Spiegelhalter. Chapman and Hall, London, U.K. pp. 1–19.

Gudmundsson, G. 1994. Time series analysis of catch-at-age observations. Appl. Stat. **43**: 117–126.

Gudmundsson, G. 1995. Time series analysis of catch-at-length observations. ICES J. Mar. Sci. **52**: 781–795.

Hilborn, R., and Liermann, M. 1998. Standing on the shoulders of giants: learning from experience in fisheries. Rev. Fish Biol. Fish. **8**: 273–283.

Hilborn, R., and Mangel, M. 1997. The ecological detective: confronting models with data. Princeton University Press, Princeton, N.J.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

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Kimura, D.K., Balsiger, J.W., and Ito, D.H. 1996. Kalman filtering the delay difference equation: practical approaches and simulations. Fish. Bull. U.S. **94**: 678–691.

Lin, X., and Breslow, N.E. 1996. Bias correction in generalized linear mixed models with multiple components of dispersion. J. Am. Stat. Assoc. **91**: 1007–1016.

Ludwig, D., Walters, C.J., and Cooke, J. 1988. Comparison of two models and two estimation methods for catch and effort data. Nat. Resour. Model. **2**: 457–498.

Meinhold, R.J., and Singpurwalla, N.D. 1983. Understanding the Kalman filter. Am. Stat. **37**: 123–127.

Mertz, G., and Myers, R.A.1996. An extended cohort analysis: incorporating the effect of seasonal catches. Can. J. Fish. Aquat. Sci. **53**: 159–163.

Meyer, R., and Millar, R.B. 1999*a*. Bayesian stock assessment using a state–space implementation of the delay difference model. Can. J. Fish. Aquat. Sci. **56**: 37–52.

Meyer, R., and Millar, R.B. 1999*b*. BUGS in Bayesian stock assessments. Can. J. Fish. Aquat. Sci. **56**: 1078–1087.

Millar, R.B., and Meyer, R. 2000. Non-linear state space modeling of fisheries biomass dynamics using the Metropolis–Hastings within-Gibbs sampling. Appl. Stat. **49**(3).

Myers, R.A., and Cadigan, N.G. 1995*a*. Statistical analysis of catch-at-age data with correlated errors. Can. J. Fish. Aquat. Sci. **52**: 1265–1273.

Myers, R.A., and Cadigan, N.G. 1995*b*. Was an increase in natural mortality responsible for the collapse of northern cod? Can. J. Fish. Aquat. Sci. **52**: 1274–1285.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol. Appl. **7**: 91–106.

Myers, R.A., Bowen, K.G., and Barrowman, N.J. 1999. Maximum reproductive rate of fish at low population sizes. Can. J. Fish. Aquat. Sci. **56**: 2404–2419.

Pella, J.J. 1993. Utility of structural time series models and the Kalman filter for predicting consequences of fishery actions. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse, D.M. Eggers, R.J. Marasco, C. Pautzke, and T.J. Quinn II. Alaska Sea Grant College Program Rep. No. 93-02, University of Alaska, Fairbanks, Alaska. pp. 571–593.

Raftery, A.E. 1996. Hypothesis testing and model selection. *In* Markov chain Monte Carlo in practice. *Edited by* W.R. Gilks, S. Richardson, and D.J. Spiegelhalter. Chapman and Hall, London, U.K. pp. 163–187.

Reed, W.J., and Simons, C.M. 1996. Analyzing catch–effort data by means of the Kalman filter. Can. J. Fish. Aquat. Sci. **53**: 2157–2166.

Richards, L.J., and Schnute, J.T. 1998. Model complexity and catch–age analysis. Can. J. Fish. Aquat. Sci. **55**: 949–957.

Rose, G.A. 1997. The trouble with fisheries science! Rev. Fish Biol. Fish. **7**: 365–370.

Sangster, G.I., Lehmann, K., and Breen, M. 1996. Commercial fishing experiments to assess the survival of haddock and whiting after escape from four sizes of diamond mesh cod-ends. Fish. Res. **25**: 323–345.

Schaefer, M.B. 1954. Some aspects of the dynamics of populations important to the management of the commercial marine fisheries. Bull. Inter-Am. Trop. Tuna Comm. **1**: 25–56.

Schnute, J.T. 1994. A general framework for developing sequential fisheries models. Can. J. Fish. Aquat. Sci. **51**: 1676–1688.

Spiegelhalter, D.J., Freedman, L.S., and Parmar, M.K.B. 1994. Bayesian approaches to randomized trials. With discussions. J. R. Stat. Soc. Ser. A (Gen.), **157**: 357–416.

Spiegelhalter, D.J., Thomas, A., Best, N.G., and Gilks, W.R. 1996. BUGS 0.5: Bayesian inference using Gibbs sampling, manual (version *ii*). MRC Biostatistics Unit, Cambridge, U.K.

Sullivan, P.J. 1992. A Kalman filter approach to catch-at-length analysis. Biometrics, **48**: 237–257.

Sun, D., and Berger, J.O. 1998. Reference priors with partial information. Biometrika, **85**: 55–71.

Appendix A

This appendix contains the entire WINBUGS (BUGS for Windows, version 1.1.1) program required to implement the example. The data (tables 1A and 1B of Myers and Cadigan 1995*b*) are contained in a separate data file that is loaded into WINBUGS by double clicking on the file icon. WINBUGS' menus are used to monitor and sample from the joint posterior distribution. Notes:

(*i*) All lines beginning with the character # are comments.

(*ii*) In this code, `M` is used to denote m^{+}.

(*iii*) BUGS follows the Bayesian tradition of using precision rather than variance when specifying the parameters of a distribution such as the normal or lognormal. Precision is the inverse of variance.

(*iv*) BUGS uses the symbol `<-` as the "equals" operator and uses `~` to denote "distributed as." For example, `x~dnorm(0,0.25)` denotes that `x` has a normal distribution with mean and variance equal to 0 and 4, respectively. To denote the lognormal distribution, `dlnorm` is used.

(*v*) Values `p1` through `p12` are scalar constants specifying parameters of prior distributions. Several of these constants have values that can be justified via reasonable argument or from prior belief. For example, the prior on `MeanLogM` could reflect the historical value of 0.2 used for natural mortality by setting `p3=log(0.2)`. Then, the prior for `M[a,y]` has median of 0.2. Other values can be chosen to reflect a lack of prior knowledge.

```
#State-space sequential population model for the age-structured 2J3KL cod

#data from Myers and Cadigan (1995b)

01 model cod; {
```

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```
   ########Distribution for data, I[a,y]######
02 for (a in 1:A) {
03  for (y in 1:Y) {
04   MeanLogI[a,y]<-Logq[a]+(LogN[a,y]+11*LogN[a+1,y+1])/12;
05   I[a,y]~dlnorm(MeanLogI[a,y],PrecLogI); } }

   #####Prior on mortalities, hierarchical######
06 PrecLogM~dgamma(p1,p2);
07 for(a in 1:A){ MeanLogM[a]~dnorm(p3,p4);
08  for(y in 1:Y) { M[a,y]~dlnorm(MeanLogM[a],PrecLogM); } }

   #####Cohort equations#####
09 for(a in 2:(A+1)) {
10  for(y in 2:(Y+1)) {
11   LogN[a,y]<-log(exp(LogN[a-1,y-1])*exp(-M[a-1,y-1])-
12                                        C[a-1,y-1]*exp(-0.5*M[a-1,y-1])); } }

   ######Priors on first row and column#######
13 for(y in 1:(Y+1)) {LogRecruits[y]~dnorm(p5,p6);
14   LogN[1,y]<-LogRecruits[y]; }
15 for(a in 2:(A+1)) {LogInitials[a-1]~dnorm(p7,p8);
16   LogN[a,1]<-LogInitials[a-1]; }

   ######Prior on q's######
17 for (a in 1:A){ Logq[a]~dnorm(p9,p10); }

   ######Prior on precision of log(I)######
18 PrecLogI~dgamma(p11,p12); }
```

Appendix B

Myers and Cadigan (1995*a*, 1995*b*) discovered that the relative biomass indices were correlated within years. They modeled this correlation in the form

$$\log(I_{a,y}) \sim N(\log(q_a N^I_{a,y}) + \xi_y, \sigma^2)$$

where ξ_y are independent $N(0,\phi)$. This new specification for the distribution of $I_{a,y}$ can be added to the model by inserting as line 19

```
19 for (y in 1:Y) {xi[y]~dnorm(0,p13);}
```

and changing line 04 to

```
04   MeanLogI[a,y]<-Logq[a]+(LogN[a,y]+11*LogN[a+1,y+1])/12+xi[y];
```

References for Appendices A and B

Myers, R.A., and Cadigan, N.G. 1995*a*. Statistical analysis of catch-at-age data with correlated errors. Can. J. Fish. Aquat. Sci. **52**: 1265–1273.

Myers, R.A., and Cadigan, N.G. 1995*b*. Was an increase in natural mortality responsible for the collapse of northern cod? Can. J. Fish. Aquat. Sci. **52**: 1274–1285.

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A multivariate approach for defining fishing tactics from commercial catch and effort data

Dominique Pelletier and Jocelyne Ferraris

Abstract: Fishing tactics correspond to fishing practices at the scale of the fishing operation. In the case of mixed fisheries, they are defined as a combination of target species, gear, and fishing location, at a given time of the year. This paper proposes an approach to determine fishing tactics from the analysis of commercial catch and effort data. The approach is based on typologies of fishing operations involving multivariate descriptive methods like factorial analyses and classification techniques. The obtained types of fishing operations are considered as fishing tactics. The approach is applied to two contrasting examples, a small-scale Senegalese fishery and a Celtic Sea fishery that operates at a larger scale. Catch per species and fishing effort are computed per tactic. Results emphasize the importance of fishing location and seasonal effects for characterizing fishing tactics.

Résumé : Les tactiques de pêche correspondent aux pratiques de pêche à l'échelle de l'opération de pêche. Dans le cas des pêcheries composites, elles se définissent comme la combinaison d'une ou plusieurs espèce(s)-cible(s), d'un engin et d'un lieu de pêche, à une époque de l'année. Cet article propose une approche pour déterminer les tactiques de pêche à partir de l'analyse de données commerciales de captures et d'effort de pêche. L'approche est basée sur des typologies d'opérations de pêche mettant en jeu des méthodes descriptives multivariées comme des analyses factorielles et des techniques de classification. Les types d'opérations de pêche obtenus sont considérés comme des tactiques de pêche. L'approche est appliquée à deux exemples contrastés, une pêcherie artisanale sénégalaise, et une pêcherie hauturière de la mer Celtique. La capture par espèce et l'effort de pêche sont calculés par tactique. Les résultats montrent l'importance du lieu de pêche et des effets saisonniers dans la caractérisation des tactiques de pêche.

Introduction

Studies on fisheries dynamics and stock assessment have traditionally focused on the resource, leaving aside the fisher component, which is akin to omitting the predator in a predator–prey system (Hilborn and Walters 1992, p. 104). However, ignoring fisher behaviour in models can result in a wrong perception of the dynamics of the fishery, in erroneous stock assessments, and finally in inappropriate management advice. This is particularly striking in multispecies multifleet fisheries, also called mixed fisheries. In such fisheries, many species are caught at once in a given area due to underlying species assemblages that reflect ecological communities. Also, there are technological interactions between the fleets (Murawski 1984), i.e. the fleets exploit the same stocks either simultaneously or sequentially, either in the same area or in segregated areas.

A fleet is often identified by the vessel and (or) crew characteristics, the gear used, and sometimes the main species targeted. However, this is unsufficient to describe fisher behaviour in that vessels of a given fleet may exhibit different fishing practices, depending on the skipper and many fluctuating factors including market conditions (e.g., see Hilborn and Ledbetter 1985). Target species may thus change in the course of the year, and for a given target species, the gear used and fishing location may also change in relation to the spatial and seasonal dynamics of corresponding populations. As a consequence, each fishing practice is likely to impact exploited stocks in a particular way. This heterogeneity of fishing practices within a given fleet precludes any attempt to accurately assess the relationship between the total fishing effort of the fleet and the resulting fishing mortality exerted on the exploited stocks. Such a relationship must be evaluated for each fishing practice. A first step is the identification of these practices for every fleet in the fishery.

Like Lewy and Vinther (1994), we believe that there are two levels at which fishing practices must be characterized, namely the fishing unit (vessel and crew) and the fishing operation. These pertain to different time scales: a few hours or a day for the fishing operation versus one or several years for the fishing unit. In this paper, we are only interested in fishing practices at the scale of the fishing operation.

The decisions made before each fishing operation should be unambiguously described by the combination of a fishing location, a gear to use, and one or several target species. Such combinations have been termed "métiers" (Biseau and Gondeaux 1988; Laurec et al. 1991; and others), "directed fisheries" (Lewy and Vinther 1994), "fishery management units" (Murawski et al. 1983), "fishing strategies" (Rogers and Pikitch 1992; He et al. 1997), and "fishing tactics" (Laloë and Samba 1991). We prefer to use this last term to reflect the decisions made before each fishing operation.

For a given fleet or fishery, identifying fishing tactics con-

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sists in determining the main types of fishing operations, i.e., constructing groups of fishing operations that correspond to similar choices in terms of target species, gear, and fishing location. Further insight into the tactics is given by additional variables like month or season.

In defining fishing tactics, the selection of fishing location is central: fishers allocate fishing effort in a given area on the basis of prior information about the profitability determined by costs and expected benefits (Hilborn and Ledbetter 1979; Sampson 1991).

Existing studies aiming at defining fishing tactics from the analysis of commercial data generally rely on applications of cluster analysis to catch per species (species composition), per fishing operation or per fishing trip. Lewy and Vinther (1994) and He et al. (1997) defined clusters of fishing trips based on the similarities between the species assemblages observed in catches. They examined in addition other characteristics of the clusters, such as fishing zone, season, vessel size, and fishing effort. Murawski et al. (1983) also analyzed species compositions to determine clusters of combinations of fishing area, depth, and month. Rogers and Pikitch (1992) used three methods to validate fishing tactics defined in an earlier study from fishers and manager interviews, with respect to types of catch profiles (relative species composition) obtained from observer data. Bertignac (1992) determined types of species composition by applying factorial analyses and classification methods to catch per unit effort (CPUE) data from Atlantic French trawlers. Auxiliary variables such as home port and vessel characteristics were used to describe changes in fishing activity over years. Hence, most existing papers focus on catch data, which might be appropriate for characterizing fishing areas (Murawski et al. 1983) or for validating predefined fishing practices (Rogers and Pikitch 1992). However, fishing tactics are not defined solely from catch composition (see above). Furthermore, target species (which is by definition tied to fisher decision) may not be accurately reflected by catch. In several papers, this was mitigated by relating the clusters with additional variables like vessel characteristics or fishing grounds (Lewy and Vinther 1994; He et al. 1997).

Ideally, fishing tactics should be explicitely characterized by target species, gear, fishing location, and time of the year. Whereas the last three are directly determined from effort data, target species could be better discriminated by analyzing jointly catch and effort data.

In this paper, we propose a methodology to identify fishing tactics from multivariate analyses of both catch and effort data with spatial and temporal references. These data as well as additional information were used to characterize the clusters obtained, with an associated statistical diagnostic of significance. In order to illustrate the wide applicability of the proposed methodology, it was applied to two contrasting examples, one from a small-scale African fishery and the other from a large-scale French fishery.

A methodology to determine fishing tactics from commercial catch and effort data

In this section, we present a methodology to construct the groups of fishing operations (i.e., the tactics) from the analysis of commercial catch and effort data. Catch and effort data are collected independently from each other. To be able to analyze them jointly, the analysis successively involves two steps: (*i*) identifying the different types of species composition from the catch data and (*ii*) identifying fishing tactics from both the types of species composition and the effort data (Fig. 1).

To obtain fishing tactics, it is necessary to have information at the scale of the fishing operation. In general, commercial catch and effort data per fishing operation consist of large data sets with many variables and many individuals (i.e., fishing operations). Variables related to catch information include numbers or tonnage caught per species, whereas variables related to effort include, among others, those related to gear, vessel, fishing location, and date. Some of these variables are quantitative, whereas others are categorical.

In addition to obtaining fishing tactics, it is also desirable to identify the values of the variables that characterize them.

One must then resort to methods that are capable of (1) providing a reduced description of large data sets, (2) analyzing relationships between variables, (3) constructing clusters of individuals, and (4) characterizing these clusters by explanatory variables. The methods must be able to handle either quantitative or categorical variables. Multivariate descriptive techniques are helpful for these purposes. In a first step, objectives 1 and 2 are attained through factorial analyses (a type of ordination technique, see below) based on a geometric representation of the data. In a second step, a classification (sometimes called clustering) is carried out on factorial coordinates to address objectives 3 and 4. The factorial analyses used in this paper are principal components analysis (PCA), two-way correspondence analysis (TWCA), and multiple correspondence analysis (MCA). PCA is a well-known technique used with quantitative variables. TWCA and MCA are equivalent techniques for categorical variables.

Analyzing this kind of data table includes selecting the relevant variables and individuals, coding the variables, applying factorial analyses and classification, and finally interpreting clusters of individuals. The whole process is in essence iterative. It will be termed "typology" in the rest of the paper.

We first present the main features of the factorial analyses and classification techniques used in the typologies, emphasizing the critical choices in using these methods and the differences between the factorial analyses used in the paper. In particular, we explain how similarities between individuals are evaluated, depending on both the type of analysis and the nature of the variables. This is crucial, since for a given data set, dramatically different clusters may be obtained in the classification step, depending on the analysis performed (and other choices such as data coding) in the first step. Because the goal of this study is to determine groups of individuals, these methods are presented from the standpoint of inertia and distances between individuals (see below). Because no such presentation could be found in the accessible literature, we give the basic algebra necessary to thoroughly understand the essence of each method in relation to the objectives of the typology. We then explain how we use factorial analyses and classification to come up with typologies of fishing operations.

Inertia and factorial analyses

A typical data set of commercial data may be framed as a data matrix X of numerical values x_{ij} with n rows and p columns, each row corresponding to an individual (e.g., a fishing operation) and each column corresponding to a variable (e.g., a variable of interest for the fishing operations). Each individual may thus be represented in the p-dimensional space defined by the p column vectors, so that the distance $d(i,i')$ between two individuals i and i' may be defined. The inertia of the data set is defined as

$$(1) \qquad I = \frac{1}{2}\sum_{i=1}^{n}\sum_{i'=1}^{n} m_i m_{i'} d^2(i,i') = \sum_{i=1}^{n} m_i d^2(i,G)$$

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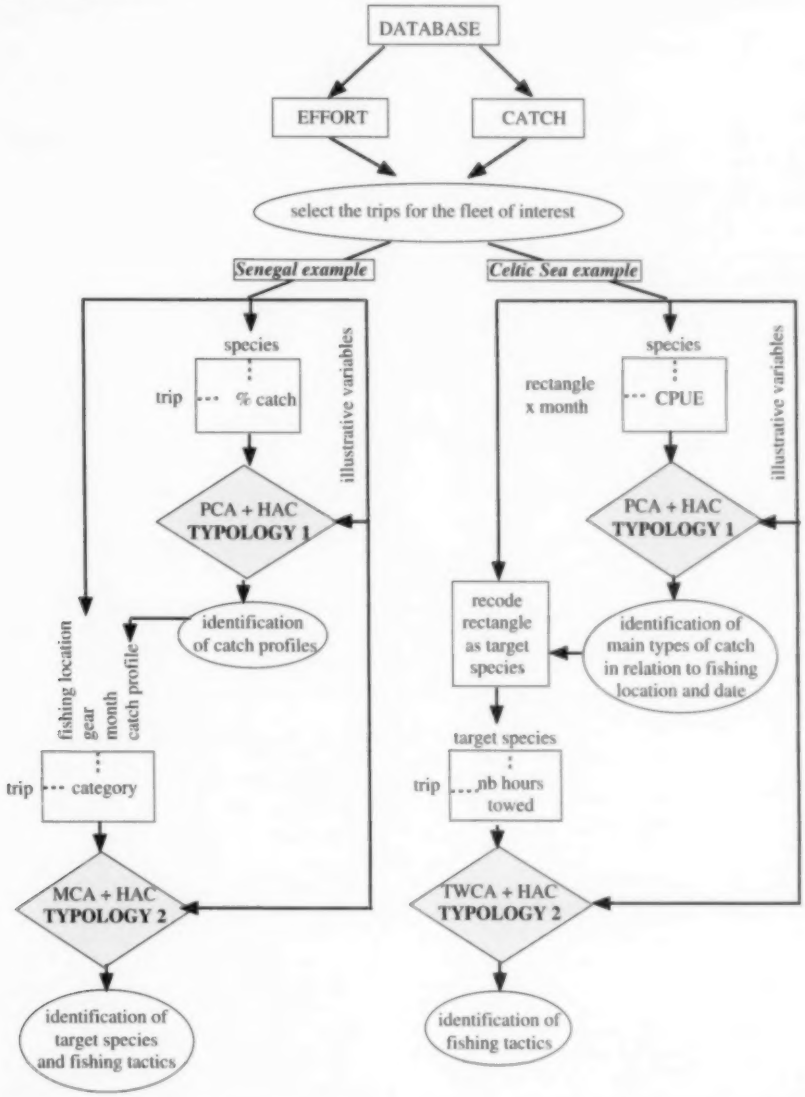
Fig. 1. Flowchart of the methodology used to obtain the fishing tactics in the two examples. In each example, two typologies are performed: (*i*) on catch data leading to approaching the target species by a categorical variable, and (*ii*) on both this categorical variable and effort data to identify fishing tactics.



where m_i and $m_{i'}$ are the weights of individuals i and i' and G is the center of gravity of the individuals, i.e., the jth coordinate of G is

$$g_j = 1/m \Sigma_{i=1}^{n} m_i x_{ij},$$

m being the sum of the individual weights. The inertia of the data set may thus be seen as a measure of the variability of the individuals around G.

Factorial analyses yield the coordinates of the individuals in the p-dimensional space where axes are ordered according to decreasing contribution to the inertia of the data set. These ordered axes are called factorial axes or sometimes ordination axes (e.g., see Rice 1985). The first axis is the one along which the n individuals are most scattered, i.e., they are best discriminated. This amounts to maximizing the inertia of the projections of the individuals on this axis. The inertia and thus the factorial axes depend on the way distances between individuals are computed, which differs according to the type of factorial analysis (see Appendix). The spanning vectors of the factorial axes are the normalized eigenvectors of a square matrix A (see Appendix), called the inertia matrix because its trace (i.e., the sum of its diagonal terms) corresponds to the inertia I of the data set defined in eq. 1. Diagonalizing A yields

$$(2) \qquad A = {}^{t}U \Lambda U$$

where U is the matrix of normalized eigenvectors and Λ the diagonal matrix of eigenvalues. Each p eigenvector being a linear combination of the initial variables, it defines a new variable, the variance of which is the corresponding eigenvalue. The inertia of the data set is then the sum of the eigenvalues.

One may calculate the coordinates of the n individuals and the p variables in the vector space spanned by the eigenvectors (see Ap-

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pendix). More generally, the coordinates of any individual for which the same p variables have been measured may be calculated (see Appendix). The coordinates of any variable that has been measured on the same n individuals could also be derived. Individuals and variables that were not used to obtain the factorial axes are called illustrative (the others are called active). They are in general useful in interpreting the results.

Factorial analyses provide a geometric representation of individuals and variables, which is easier to interpret than the initial data table. In addition, they allow reduction of the dimensions of the data table by retaining only the axes that explain up to a given part of the inertia. This feature may also be helpful in eliminating marginal effects that might blur the structure of interest in the data set. The general shape of the histogram of eigenvalues shows the gain in accounted for inertia offered by retaining a given number of axes (see the first application).

Classification

Classification techniques aim at grouping the n individuals into clusters that are both well separated and as homogeneous as possible with respect to the observed variables. Cluster homogeneity may thus be related to inertia. Consider a partition P_C of the n individuals into C clusters. Let n_c denote the number of individuals in the cth cluster. Following Huygens' classical theorem, the inertia of the data set defined in eq. 1 may be decomposed into within-cluster and between-clusters inertia:

$$(3) \quad I = I_{\text{between}(P_C)} + I_{\text{within}(P_C)} = \sum_{c=1}^{C} m_c d^2(G_c, G) + \sum_{c=1}^{C} \sum_{i=1}^{n_c} m_i d^2(i, G_c)$$

where G_c is the center of gravity of the cth cluster and $m_c = \sum_{i=1}^{n_c} m_i$ is the weight of G_c.

In this paper, we use a hierarchical ascending classification (HAC) technique in which the clusters are built by successive pairwise agglomerations of elements based on the minimum variance criterion of Ward (1963). Hierarchical techniques are useful when the desired number of clusters is not known in advance because they provide nested partitions under the form of a dendrogram. For very large data sets, hierarchical clustering is instead applied to homogeneous groups obtained in a first step from a nonhierarchical partitioning (Lebart et al. 1984, pp. 130–132). The partitioning algorithm used in the first application relies on clustering around moving centers, which belongs to K means methods (McQueen 1967).

The variation of the aggregation criterion is used to select the appropriate number of clusters by cutting the dendrogram. A break in this variation indicates that the corresponding step has agglomerated rather different entities, leading to a substantial increase in the heterogeneity of resulting clusters.

Selecting the appropriate number of clusters is not only tied to statistical or numerical considerations, but also depends on the objective of the analysis. A limited number of clusters may, for instance, be desirable when the results will serve to construct a fishery model. In contrast, one might be interested in obtaining more clusters to gain a better insight into the data set. Selecting the number of clusters should also rely on the relevance of cluster interpretation. The latter is achieved by examining the differences in means (resp. frequencies) between each cluster and the total population of individuals, for each numerical variable (resp. each category). Such differences are statistically tested using test values (Lebart et al. 1984). When the test value is significant, the mean (resp. the frequency) of the variable (resp. the category) is significantly higher or lower in the cluster compared with the total population of individuals, and the variable (resp. the category) is said to be characteristic of the cluster.

A combined approach to obtain typologies

In this paper, each typology is obtained from a factorial analysis (PCA, TWCA, or MCA) followed by a classification on the factorial coordinates. There are several reasons for performing a classification after a factorial analysis. First, the factorial axes and the projections of individuals on these axes may be difficult to interpret. For instance, the third axis describes proximities between individuals that are residuals with respect to the first two axes. Second, some individuals may be very influential in the construction of axes and drive the results in an excessive manner. Classification palliates each of these drawbacks because it summarizes the data set in a way that is much easier to interpret than individual projections. Besides, it is to some extent more robust to marginal individuals than factorial analysis because of the iterative algorithms used.

Conversely, there are also several reasons for performing a factorial analysis before a classification. Whatever the initial variables, it provides a geometric description of the individuals, the variables, and the relationships between them that is helpful in exploring the structure of the data set. For instance, illustrative individuals are more easily allocated to a cluster through their factorial coordinates. Also, factorial coordinates are needed to project clusters on factorial axes, which facilitates their interpretation.

Our results were obtained from the software SPAD.N (CISIA 1996), which is specifically designed for multivariate descriptive analyses and allows the linkage of several kinds of analyses in a user-friendly manner. However, the factorial analyses (PCA, TWCA, and MCA) and the classification techniques quoted in the paper are available in most statistical software, including Splus and SAS.

The artisanal fishery of Senegal

Background to the fishery

The small-scale marine fishery of Senegal is among the most important artisanal fisheries of West Africa, with, for example, 35 000 fishers and 5661 canoes in 1992. The fishery's catch (300 000 t) contributes to more than 70% of the nation's annual landings. This tropical fishery takes place in an upwelling area where most demersal and pelagic species migrate and spawn during the upwelling season due to increased primary and secondary production. Fisher behaviour varies with the seasonality of fish catchability, so that fishers change gears, target species, and fishing locations during the year. The catch is characterized by a great number of species dominated by clupeoids (*Sardinella aurita*, *Sardinella maderensis*); other species caught include sea bream (*Pagellus bellottii*, *Sparus caeruleostictus*, *Dentex* sp.), catfish (*Arius* sp.), groupers (*Epinephelus aeneus*, *Epinephelus goreensis*), cephalopods (*Octopus vulgaris*, *Sepia officinalis*), sharks, and rays. The fleet consists of canoes with length ranging from 3 to 20 m. Most canoes are motorized with an engine power ranging from 6 to 29 kW. Crew size lies between one and 12. Fishers use 24 categories of gear belonging to five main types: pursing nets, drifting gill nets, set nets, hooks and lines, and beach seines. Trip duration is limited to 12 h except for canoes equiped with an ice box, which may fish further from home port. A given canoe may have several gears and resort to different fishing practices, depending on target species.

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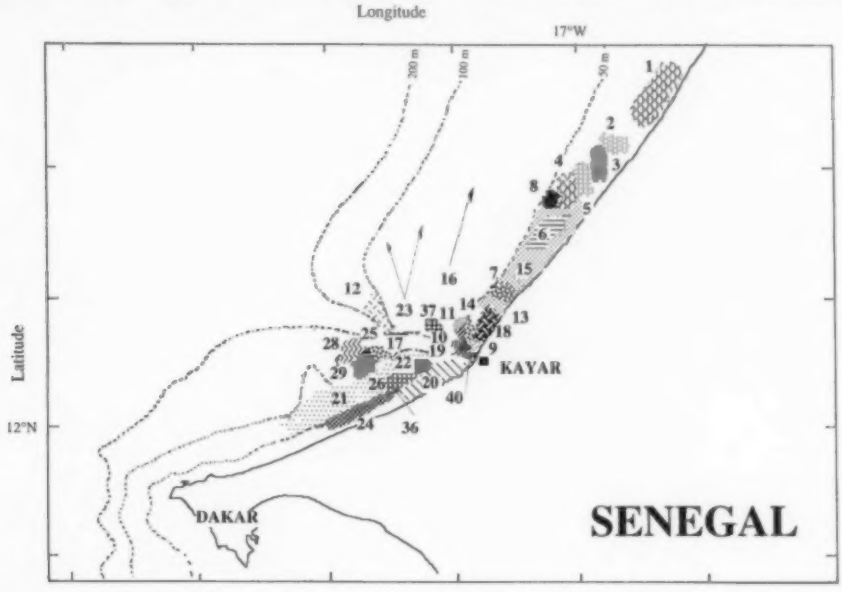
Fig. 2. Location of the small-scale Senegalese fishery. Fishing locations are indicated by shaded areas or arrows (directions).



Data

The data collection system includes a census for fishing units carried out twice a year and catch and effort sampling throughout the year. A census of canoes by fishing village and by gear provides an exhaustive description of fishing units. The data used here were obtained from the survey undertaken in 1992 by the Centre de Recherches Océanographiques de Dakar-Thiaroye (CRODT) in the port of Kayar north of Senegal (Fig. 2).

Sampling for catch and effort data follows a stratified scheme, with one stratum per region, fortnight, and gear. Fishing effort is estimated by the number of trips per gear and working day. Regarding catch, approximately 10% of fishing trips are sampled in each stratum, based on a two-stage scheme with days as primary units and landing canoes as secondary units. For each sampled fishing trip, fishing location, trip duration, depth, crew size, and species composition of catch are recorded. Every fishing location (Fig. 2) has a name that reflects either the geography or a characteristic of the location (e.g., a direction where to fish). In Kayar in 1992, canoes were censused in April (491 crafts) and in November (301 crafts). Corresponding total effort was estimated to be 80 233 fishing trips, of which 6892 were sampled for catch. Among these trips, 5521 belonged to the hand-line category. During those trips, 28 fishing locations were visited and 121 species were caught. For these canoes, gear was coded in three categories: G1 for canoes with no engine, G2 for canoes with engine power ≤9 kW, and G3 for canoes with engine power >9 kW.

Identification of catch profiles

A data matrix was built with fishing trips using hand line as individuals and catch per species as variables (Fig. 1). Only fishing trips with nonzero catch were considered, i.e., 5427 trips. For each trip, absolute catch was transformed into a catch profile (i.e., a relative species composition) by dividing each catch per species by the total catch. This removed the differences in catch levels between trips, which are often linked to both the time of the year and to crew size. Data were then log transformed to symmetrize their distribution. A nonnormalized PCA was run in order to account for differences in abundances between rare species and dominant species. All the factorial axes were retained, and a HAC of the factorial coordinates led to a partition into eight clusters that explained 44.4% of the inertia of the data. For each cluster, one species was found to be highly characteristic, and several other species showed significant test values. On the whole, 35 species out of 121 exhibited a highly significant test value. Unlike cluster "Octopus," which was characterized by a single species (*Octopus vulgaris*), the other clusters were generally determined by several species. The cluster was then named after the dominant species or by a term describing the species assemblage. For instance, the cluster "Grouper" was characterized by several species of *Serranidae* associated with other demersal species. The largest and best-discriminated cluster was "Sea bream" (1702 trips), which was characterized by the species *Pagellus bellottii*. Clusters Octopus (816 trips) and Grouper (825 trips) were characterized by species living on rocky substrate, whereas clusters "Large-eye dentex" (331 trips) and "Deepwater group" (331 trips) were found to correspond to deepwater species. Clusters "Sailfish" (252 trips), "Goatfish" (323 trips), and "Warmwater group" (868 trips) were tied to species mainly caught during the warm season.

Each cluster corresponds to one type of catch profile, which was then considered as a categorical variable termed "catch profile." Each trip with a nonzero catch was thus assigned a category of catch profile.

In the absence of fisher interviews, a catch profile provides information about target species. We will relate it to

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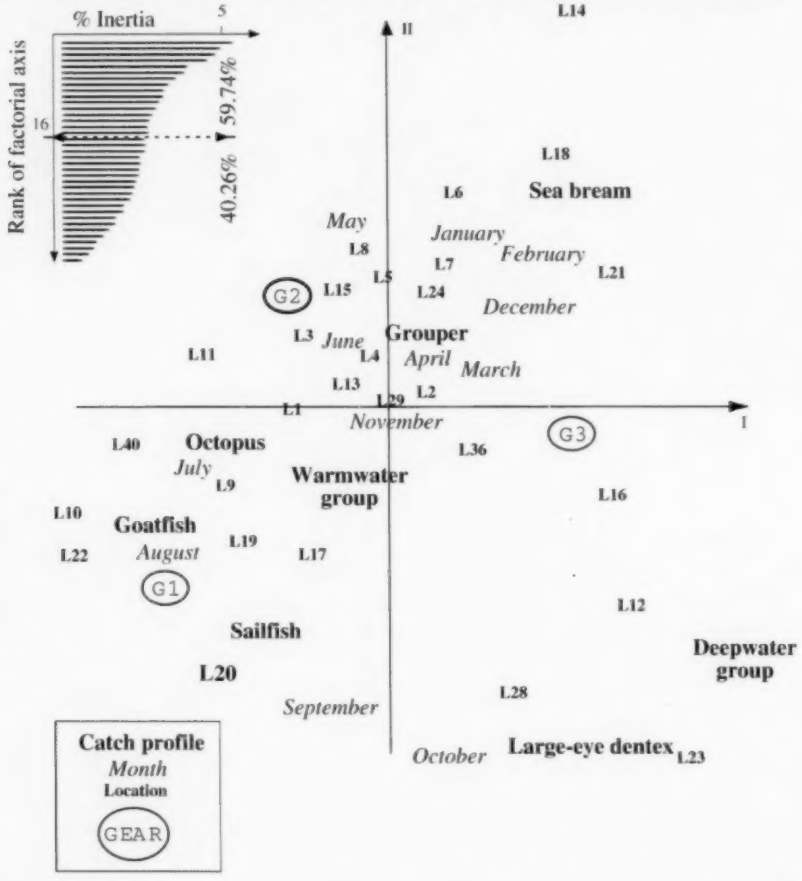
Fig. 3. Representation of active categories on the first two factorial axes of the second typology in the Senegal example. This shows the relationships between the 51 categories of the four variables used to describe the fishing operations. Different fonts are used to report each variable (see box at the bottom of the plot). The inertia accounted for by each axis is shown in the inset. The arrow indicates the 16th value, the cumulated inertia for the 16 first axes being 59.74%. L1, L2, etc., are fishing locations (see Fig. 2).



fishing conditions (depth, location, etc.) and (or) fishing gear in order to complete the identification of target species and to define fishing tactics.

Identification of fishing tactics

For each trip, fishing activity was described by a number of variables (location, gear, month, crew size, and depth). These variables were all found to be significantly correlated with each other and with catch profile (probas of the χ^2 test, <0.001). In the following, we retained only those variables that appear explicitly in the definition of a fishing tactic, i.e., target species approximated through catch profile, fishing location, gear. and month.

Our aim was to examine (*i*) which categories of these variables were associated in the definition of fishing tactics and (*ii*) which similarities between fishing trips might be evidenced with respect to these variables. For this purpose, an MCA was applied to the data matrix built with the 5427 fishing trips as individuals and the four categorical variables: catch profile, fishing location, gear, and month (which resulted in 51 categories) (Fig. 1). Ninety-four fishing trips with no catch profile (because of a null catch) were treated as illustrative individuals. In this case, the catch profile was coded as an additional category corresponding to missing data.

The histogram of eigenvalues (Fig. 3, inset) shows that the first four axes predominate (20.4% of the inertia), with a break in the decrease of the inertia accounted for by the axes. These four axes are considered as relevant to depict the relationships between the categories. As an illustration, the first two factorial axes (Fig. 3) showed a close link between deep locations (L23, L12, and L28) on the one hand and the catch profiles Deepwater group and Large-eye dentex on the other. Sea bream and Grouper were related to the cold upwelling season (December–May), while Sailfish, Octopus, Goatfish, and Warmwater group appeared to be associated with the warm season (July–October). Motorized canoes (G2 and G3) were opposed to canoes with no engine (G1) on the second factorial axis.

In a second step, a classification was applied to the factorial coordinates obtained from the MCA so that clusters of fishing trips could be obtained on the basis of the relationships between the four categorical variables. To eliminate marginal effects that might blur the structure of the data set,

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Fig. 4. Fishing effort in number of trips as a function of month (*a*) per gear and (*b*) per tactic in the Senegal example.

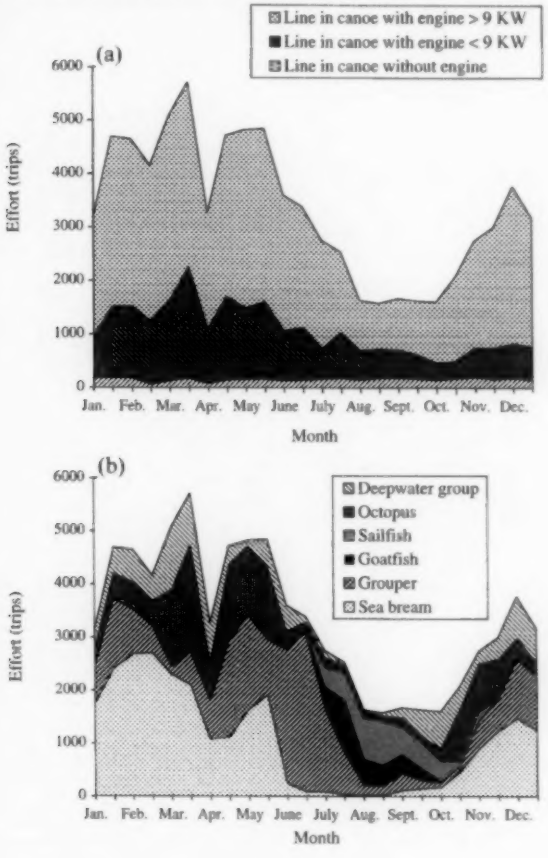
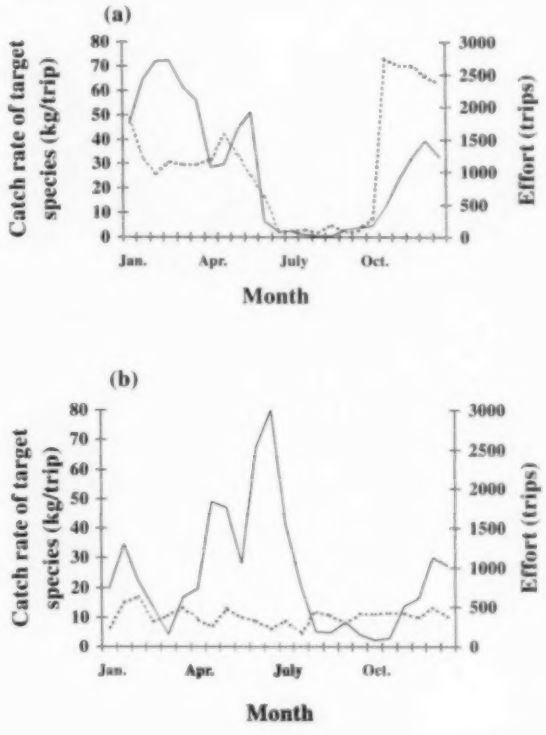


Fig. 5. Effort per tactic and average catch rate of the target species for the tactics (*a*) Sea bream and (*b*) Grouper in the Senegal example.



only the first 16 factorial axes (60% of the inertia; Fig. 3, inset) were retained for classification. A two-step classification led to seven clearly identified clusters. The 94 illustrative individuals were allocated to the nearest cluster on the basis of their factorial coordinates. This analysis assigned to the same cluster (i.e., to the same tactic) the trips that exhibited similar categories of the active variables (catch profile, fishing location, gear, and month). These fishing tactics were then interpreted from both the active and illustrative variables (Table 1). Clusters 6 and 7 were arbitrarily pooled into a single tactic (Deepwater group in Table 1) because they appeared similar for all the variables except season. Six tactics were finally retained (Table 1). The most characteristic species of the illustrative variable catch per species is assumed to represent the target species of each tactic. Fishing effort was calculated per gear (Fig. 4*a*) and per tactic (Fig. 4*b*). Total effort per fortnight was extrapolated from the sample using a raising factor per gear that is the total number of sampled fishing trips divided by the total estimated effort per gear. The decrease in fishing effort during the warm season only appears for the tactics Sea bream and Octopus in June,and Grouper in August. Catch rates per species may also be calculated for each tactic (Fig. 5). For the target species of the tactic, they are in general larger than catch rates calculated per gear. Note that for motorized crafts, catch rates are quite similar irrespective of engine power (G2 and G3). For the Sea bream tactic, the catch rate of *P. bellottii* follows the same trend as the fishing effort. The tactic strongly targets this species with an average catch rate of 39.9 kg per trip (±33.2 kg) for *P. bellottii* versus an overall catch of 53.7 kg per trip (±39.1 kg). For the Grouper tactic, catch rate and effort show different temporal patterns. Effort increases markedly from November to February and from April to July in relation to the immigration of *E. aeneus* in the region. The catch rate for this species thus remains relatively constant throughout the year. Unlike the Sea bream tactic, the Grouper tactic targets several species of commercial interest, so that the catch rate of the target species is 9.4 kg per trip (±13.9 kg) versus an overall catch of 34.5 kg per trip (±36.9 kg).

The Celtic Sea groundfish fishery

Background to the fishery and data

The Celtic Sea is a continental sea that is bounded on its eastern and northern sides by France, United Kingdom, and Ireland (Fig. 6). The main commercial species fished in this area include Atlantic cod (*Gadus morhua*), Norway lobster (*Nephrops norvegicus*), whiting (*Merlangius merlangus*), hake (*Merluccius merluccius*), monkfish (*Lophius budegassa*, *Lophius piscatorius*), megrim (*Lepidorhombus whiffiagonis*), and rays (in particular, cuckoo ray (*Raja naevus*)). Some

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Table 1. Characterization of the tactics obtained from the classification of fishing trips based on the four categorical variables.

Catch profile (tactic name in bold)	Active variable				Illustrative variable		
	Cluster size	Gear	Location (Fig. 2)	Month	Catch per species (target species underlined)	Depth (m)	Crew size
1. Sea bream	1490 (24)	G2, G3	L14, L6, L13, L21, L7	Feb., Jan., Mar., Dec., Nov., May	*Pagellus bellottii* *Decapterus rhonchus* Brachydeuterus auritus *Dentex canariensis*	25–50	4, 3, 5
2. Grouper Warmwater group	1321 (15)	G3	L2, L24, L5, L8, L15, L17	June, July, Apr.	*Epinephelus aeneus* Sparus caeruleostictus *Pomadasys incisus* *Epinephelus goreensis* Pomatomus saltator *Plectorhynchus mediterraneus* Epinephelus gigas	10–25 and 75–120	3, 2
3. Goatfish	339 (2)	G1	L15, L40, L10, L11	July, Aug., Sept.	*Pseudupeneus prayensis* *Diplodus vulgaris* Pomadasys rogeri *Sparus caeruleostictus* Plectorhynchus mediterraneus	10–50	1, 2
4. Sailfish Warmwater group	449 (13)	G2	L20, L13, L7	Aug., Sept., July, Oct.	*Istiophorus platypterus* *Scyris alexandrinus* Coryphaena hippurus *Brachydeuterus auritus*	25–75	2
5. Octopus Warmwater group	1112 (39)	G1	L9, L11, L40, L10, L17	Mar., Oct., May, Nov., Apr.	*Octopus vulgaris*	<25	1
6. Deepwater group	716 (1)	G3	L16, L23, L21, L17	Mar., Dec., Apr., Oct., Sept., Feb., Jan.	*Dentex* spp. *Branchiostegus semifasciatus* Brotula barbata *Scorpaena* spp. *Merluccius senegalensis* Centrophorus spp. *Trachurus trachurus*	>75	4, 5

Note: Only characteristic variables or categories are reported. All variables are ranked by decreasing order of significance as given by their test value (not reported). The tactic is named after the most characteristic category of the variable catch profile (bold). The target species is assumed to be the most characteristic category of the variable catch per species (underlined). In the second column, the number of illustrative individuals assigned to the cluster (i.e., the tactic) is reported in parentheses. In the gear column, G1 corresponds to no engine, G2 corresponds to engine power <9 kW, and G3 corresponds to engine power >9 kW. Depth is coded in seven categories, but only characteristic depth ranges are reported for clarity.

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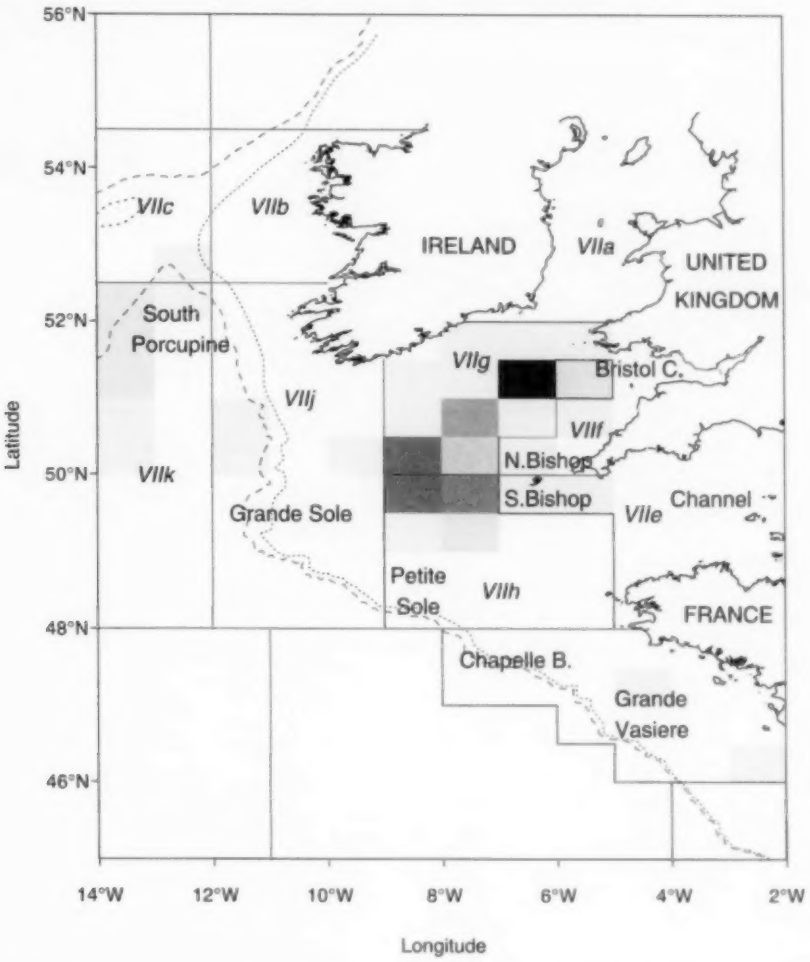
Fig. 6. Location of Celtic Sea with traditional names of fishing locations and annual allocation of fishing effort (number of hours towed) for the Nephrops tactic. Shaded areas indicate the amount of fishing effort spent by rectangle. The grey level is proportional to the number of hours towed.



other species are also caught in large quantities, like haddock (*Melanogrammus aeglefinus*), European ling (*Molva molva*), pouting (*Trisopterus luscus*), squids (*Loligo forbesii*, *S. officinalis*), red gurnard (*Aspitrigla cuculus*), and pollack (*Pollachius pollachius*). A large part of the catch is taken by a French fleet of medium-sized (18–25 m long) bottom trawlers that operate all year round, making trips of about 12 days.

Effort data consist of logbooks, whose provision is compulsory for stocks under European Union management. Logbooks indicate fishing locations at the scale of International Council for the Exploration of the Sea (ICES) statistical rectangles, i.e., zones of 1° longitude per 0.5° latitude (about 30 square nautical miles in this area), as well as the fishing effort allocated in each rectangle in hours towed. Catch data are provided by the automatic census of sales on the market hall. They include the weight and value of the catch per species for each sale of each vessel. Because the vessels sell their catch upon each return to the port, both the effort per rectangle and the total catch (per species) are known for each trip of a given vessel. Unfortunately, the catch per rectangle is only known if the vessel fished exclusively within a single rectangle (single-rectangle trip). The data used in this analysis pertain to 265 vessels exploiting the Celtic Sea in 1993, corresponding to 5262 trips.

Identification of target species in relation to fishing location and season

As in the Senegal example, target species are not known directly from the data, since only species caught are available in the data. If we assume that skippers select fishing location depending on target species, the latter should be deduced from the species composition that characterizes the selected fishing location at a given time of the year. At this stage, each statistical rectangle for a given month was thus put into correspondence to a species composition that was likely to be obtained when fishing there at that time. This most likely species composition was supposed to be the set

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Table 2. Characterization of the clusters obtained from the analysis of the mean CPUE per rectangle–month.

Cluster name	Cluster size	Typical catch per species	
		Abundant species	Rare species
Monkfish–rays	103	*Lophius* sp., *Raja naevus*, *Raja circularis*	*Nephrops* (no), gadoids
John dory – cuttlefish	23	*Zeus faber*, *Mullus*, *Sepia*, *Loligo*, *Aspitrigla cuculus*, Mustelus, *Raja montagui*	*Nephrops* (no)
Bass–gurnard	3	*Dicentrarchus labrax*, *Eutrigla gurnardus*, *Sepia*, *Mullus*	
Nephrops	73	*Nephrops*, *Lepidorhombus*, *Glyptocephalus cynoglossus*, *Squalus acanthias*, *Raja batis*	*Raja naevus*, *Lophius*, *Loligo*, *Sepia*, *Aspitrigla cuculus*, *Raja circularis*, *Zeus faber*
Rays	6	*Raja clavata*, *Raja montagui*, *Psetta maxima*, Rajidae, *Conger conger*, *Solea vulgaris*	
Gadoids	26	*Gadus*, *Merlangius*, *Melanogrammus*, *Microstomus*, *Merluccius*, *Pollachius virens*, *Pollachius pollachius*, *Molva*, *Pleuronectes*	*Lophius*, *Nephrops*, *Raja naevus*, *Lepidorhombus*, *Raja circularis*
Pouting–gurnard	11	*Trisopterus luscus*, *Aspitrigla cuculus*, *Trigla lucerna*, *Scyliorhinus canicula*, *Scyliorhinus stellaris*, *Microstomus*, *Loligo*, *Conger conger*, Rajidae, *Scophtalmus*	*Lepidorhombus*, *Lophius*, *Raja circularis*

Note: Species are ranked from the more to the less typical. The cluster is named after the most typical species composition. "Abundant" means that the species is typically caught in the class, "rare" means the reverse, and "no" in parentheses means that the species is totally absent from these rectangle–months. The species name is omitted when there is no ambiguity.

of average CPUE per species that could be computed over all the catch realized in this rectangle during that month. However, it was necessary to restrict the analysis to those trips during which only one rectangle was visited (single-rectangle trips). Otherwise, catch and rectangle could not be unambiguously matched. In 1993, this corresponded to approximately 30% of the trips. The data relative to these trips were reformatted into a table of 534 individuals (the available combinations of statistical rectangles and months) and 63 variables (the species) where each cell value was the mean CPUE of a species calculated over all trips corresponding to a given rectangle–month (Fig. 1). Only 53 species were considered as active variables. A normalized PCA and an HAC were successively carried out on this data table so as to obtain a typology of rectangle–months based on species composition. This typology thus tended to group rectangle–months with similar species abundances. Normalization implies that rare species were given the same weight as abundant species. The dendrogram (not reported) showed seven well-separated clusters that explained 27% of the inertia of the data set. This partition was considered as satisfying because it was aimed at identifying the main species compositions and not at explaining the whole variability of the data set. The 63 species were then used to characterize the clusters. A species was assumed to be typical of a given cluster if, in addition to a significant positive test value, it corresponded to nonnegligible CPUE in the fishery or if it was known through prior knowledge to be a target species. Clusters were named after their typical species composition. For each cluster, several species were found to be typical (Table 2). Most of the typical species (see Table 2) are known as target species, whereas others are merely incidental catch often associated with the target. Four clusters correspond to traditional target species of the Celtic Sea: monkfish, *Nephrops*, rays, and gadoids, while the remainder correspond to coastal stocks. Clusters were also characterized by zero or low abundances for some species that exhibited significant negative test values. To a large extent, the clusters of rectangle–months reflected the seasonal evolution of the spatial distribution of the exploited stocks. For instance, the cluster "Rays" was mainly found in winter in the northern Celtic Sea. The cluster "Gadoids" was marked in September in the northern Celtic Sea, in relation to high abundances of whiting. In contrast, the cluster "Monkfish " was tied to the continuous presence of monkfish in the southern Celtic Sea throughout the year.

Identification of fishing tactics

In a second step, data per trip were considered to determine fishing tactics. Note that the gear was the same for every trip; therefore, fishing tactics were to be defined from the combination of target species, fishing location, and time of the year.

For each trip, the date and the fishing effort per rectangle were known, so that the previous typology could be used to recode fishing effort per rectangle at a given date as fishing effort per target species. Of course, this was possible only for the trips that visited rectangles at dates for which the corresponding rectangle–month belonged to a cluster of the previous typology, which amounted to 2493 trips out of 5262 initial records. A data matrix of 2493 individuals and seven categories of the active variable, one for each target species, was thus constructed; each element of the matrix was the fishing effort spent on a given target species during a given trip (Fig. 1). A TWCA was found to be appropriate for analyzing associations between the categories of the target species variable. In addition, unlike a PCA, TWCA removes the differences in total fishing effort between trips because trips are analyzed using the proportion of the trip's effort per target species (and not the total effort per target species) (see z_{ij} in the Appendix). An HAC on the resulting factorial coordinates yielded a partition of fishing trips into nine clusters, which was considered to be satisfactory, given the available data (Table 3). This typology tended to assign to the same cluster (i.e., the same tactic) the fishing trips that showed similar allocations of fishing effort between the categories of the active variable in the TWCA (i.e., the target species). This variable was used both as active variable in

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Table 3. Characterization of the clusters of trips obtained from the analysis of fishing effort per target species.

Cluster (tactic)	Cluster size	Target species	Effort (%)	Typical fishing location(s)	Typical time of the year and effort allocation in %
1	776	**Nephrops**	98	North and central CS, South Porcupine, Grande Vasière	80% from March to August
2	22	**Rays**	46	Northern CS, Irish Sea (VIIa)	From November to January
		Nephrops	38		
		Gadoids	9		
3	76	**Gadoids**	49	Northeastern CS, Bristol Canal	64% from February to May, 25% in September
		Nephrops	40		
4	136	**Gadoids**	96	Northeastern CS, Bristol Canal	59% from February to June, 22% in August and September
5	76	**Rays**	97	Northeastern CS, Irish Sea	From September to February
6	220	**John dory – cuttlefish**	93	Eastern CS, western channel (VIIe), southern CS, Grande Vasière	50% from November to December, 39% from January to June
7	529	**Monkfish**	97	Southern CS, Petite Sole, Chapelle	Seasonality not marked
8	490	**Pouting–gurnard**	98	Northern Bishop, eastern channel	Seasonality not marked
9	150	**Bass–gurnard**	97	Southern Bishop	95% from December to March

Note: Each tactic is named after the most characteristic target species (bold). Fishing locations are given in Fig. 6. CS denotes Celtic Sea stricto sensu, i.e., ICES divisions VIIg and VIIh.

the TWCA and to characterize the clusters. The target species of a tactic was assumed to be the most characteristic target species of the corresponding cluster. To characterize the clusters of trips, the statistical rectangles visited during the trip were coded as binary variables (presence–absence) and the months were considered as a categorical variable.

Except for clusters 2 and 3, each fishing tactic was strongly characterized by a single target species (Table 3). Fishing tactics with the same target species might differ by fishing locations and (or) time of the year, like tactics 2 and 5 or tactics 3 and 4. Some tactics exhibited a strong seasonal effect, like tactics 1, 6, and 9, whereas others were more evenly practiced all year long, like tactics 7 and 8. In terms of fishing effort, the main tactics were "Nephrops," "Monkfish," and "Pouting–gurnard," each of which represented 35, 24, and 15% of total fishing effort, respectively. Only the Nephrops and Monkfish tactics were typical of the Celtic Sea per se, the Pouting–gurnard tactic focusing more on the channel area (Fig. 6). The Nephrops tactic appeared to be largely confined to the northern Celtic Sea (Fig. 6), whereas the Monkfish tactic was typical of the southern Celtic Sea. The species compositions of catch for these three main tactics illustrate the difficulty of defining target species from realized catch (Fig. 7): even for a given tactic, catch composition was always a balanced mixture of several species. In 1993, the catch of the Nephrops tactic comprised only 30% *Nephrops*, and the catch of the Monkfish tactic only 30% monkfish. The catch of the Pouting–gurnard tactic was made up of 12% pouting and 14.5% gurnards but also 24% cuttlefish and 11% long-finned squid. However, cuttlefish was not found to be characteristic of the tactic because of a high variability in catch among the trips in the tactic.

Discussion

Fishing tactics

The two examples presented contrasted from several standpoints. On the one hand, the Senegal fishery is artisanal, with fishers going out fishing for 1 day at most. Many gears are used. There is no compulsory declaration system; hence, data collection was designed and implemented by scientists. Fishing locations are identified through their names, but not georeferenced. On the other hand, the French Celtic Sea fishery is industrial, with fishers going out fishing for approximately 12 days. Only one gear is used. The data collection system relies on compulsory logbook declarations of effort per trip and automatic census of catch per sale on the market hall. Fishing locations are identified by ICES statistical rectangles.

Despite these differences, a similar methodology was applied in both examples to define fishing tactics as a combination of target species, gear, fishing location, and time of the year.

In the literature reviewed, target species were determined only from catch data. In this paper, target species were determined using jointly species composition, gear, fishing location, and time of the year. Target species obtained in this way should be closer to reality, since fishing location, unlike catch, is almost totally dependent on fisher decision. Hence, fishing location appeared to be central in defining fishing tactics. In the Celtic Sea example, catch and effort data could be linked through the typology of rectangle–months.

Because catch is highly variable from one trip to another, data analysis had to average out these fluctuations. In the Senegal example, individual catch profiles were summarized into only eight types. In the Celtic Sea example, the typology of rectangle–months was based on average CPUE, which smoothed out trip variability at that scale, and was more likely to provide an accurate picture of the spatio-temporal distribution of species abundance. Although individual trip variability was averaged out to determine target species, it was taken into account when obtaining fishing tactics, since these are groups of individual fishing trips.

Data collection

From the data collection standpoint, the determination of

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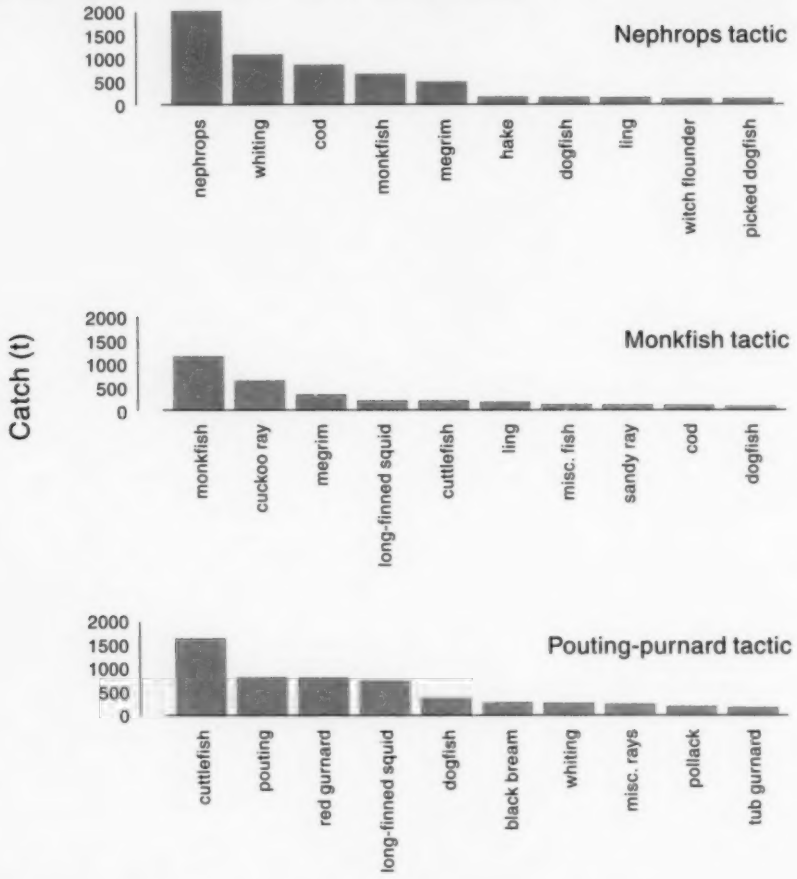
Fig. 7. Catch per species for the three main tactics found in the Celtic Sea fishery.



fishing tactics could be improved if fishing locations were more precisely defined, e.g., using positioning systems and appropriate databases. In the Senegal example, fishing locations were loosely defined. In the Celtic Sea example, effort location was not known at scales smaller than the rectangle, and most important, catch was not even known per rectangle for 70% of the trips. In these examples, like in many fisheries, data collection systems provide no information at the scale of the fishing operation, so that the obtained fishing tactics are types of fishing trip rather than types of fishing operation. In the Senegal example, fishing trips were so short that it is likely that only one fishing tactic was used during a trip, which may not be the case in the Celtic Sea example. In the latter example, two tactics appeared to be composite, with a fishing effort allocated between two or three target species, actually reflecting the fact that several tactics were used during corresponding trips.

The results might also be helpful in adapting sampling designs for commercial data in that more pertinent strata may be constructed, sampling effort may be reallocated between strata, or even information coding may be revisited. For instance, in the Senegal case, initial gear coding was not precise enough to reflect the diversity of the types of hand lines used in the fishery.

A common methodology

The approach proposed in this paper aimed at determining a few fishing tactics from an initial large number of data, i.e., many individuals (the trips) and many variables (catch and effort data). The proposed methodology relies on two successive typologies, where the cluster number of the first one serves as a categorical variable in the second one, allowing the joint analysis of catch and effort data. Each typology consists in a factorial analysis followed by a classification. On the one hand, factorial analyses provide a geometric representation of the individuals and variables that helps in exploring the structure of the data set. Selecting a limited number of ordination axes enables one to retain only the variability of interest for the classification step. In addition, clusters can be projected on these axes to facilitate their interpretation. On the other hand, classification techniques summarize the information contained in the retained axes and yield the clusters of individuals. Both factorial analyses and classification techniques may utilize either quantitative or categorical variables. Furthermore, both types of variables may be analyzed jointly in the classification step, owing to the use of factorial analyses that provide a unified representation of individuals through factorial coordinates.

These techniques accept illustrative variables that do not

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contribute to the ordination axes but that might still be of interest in describing the axes or characterizing the clusters. In the Senegal example, the tactics (i.e., the clusters) were named after the catch profile that was an active variable, but the target species of each tactic was assumed to be the most characteristic catch per species of the cluster, i.e., an illustrative variable. For the trips of this tactic, the catch of the target species was thus higher and less variable than for the other trips.

Similarly, the techniques used accept illustrative individuals that might otherwise distort the analysis as active individuals, so that they can still be projected on axes or allocated to clusters. Also, individuals with missing information may be allocated to clusters on the basis of other variables, e.g., the trips with null catch in the Senegal example

As much as possible, objective criteria were proposed at each stage of the analysis. These are mostly based on inertia, helping, for instance, in selecting the number of axes to be retained in the factorial analyses or the number of clusters in the classification. Test values allow ranking of the variables that describe the clusters according to how well they characterize them. Both negative and positive values are interesting for this purpose. However, there still remains a part of subjectiveness in the analysis. Data coding is of primary importance; data might be transformed into logs, percentages, or otherwise. The distance might rely on normalized variables or not, on the Euclidian distance, or on the χ^2 distance, leading to different weightings of the variables and individuals. Such choices are guided at the same time by the objectives of the analysis, by the structures of the data set, and by the relevance of the results with respect to prior knowledge of the fishery. For instance, in the first typology of the Senegal example, a normalized PCA led to a dendrogram (in the HAC) from which it was difficult to select a pertinent number of clusters. A clear partition could only be seen after performing a nonnormalized PCA. In contrast, a normalized PCA led to a satisfying dendrogram in the first typology of the Celtic Sea example. Like any modelling approach, these typologies are in essence an iterative process going back and forth between the analysis and the results.

Recall that the techniques used do not require any a priori assumptions about the distribution of the variables. The results are only descriptive for the data set; no inference can be drawn from the extrapolation of results to the whole population sampled.

Last, note that fishing tactics appear as a combination of several variables, possibly categorical variables. Such objects are termed "symbolic" objects in statistics. Techniques were recently developed to analyze this type of information (Diday et al. 1996). They might prove useful in this kind of study.

Perspectives

Fishing tactics reflect simultaneously the decision of the fisher (target species, gear, location, and time of the year) and the results of that decision (the catch). Hence, a typology of fishing tactics provides a synthetic representation of the fishing operations, which is not an objective per se. It is rather a necessary step in understanding the dynamics of mixed fisheries. A typology is useful for several purposes. First, it yields a basis to partition the fleet into components that bear similar impacts on the resources. Catch per species and effort may be calculated per component. In this respect, it might help in evaluating management measures that target particular fishing activities. In practice, this kind of measure has been traditionally assessed by resorting to fleet partitions based on measurable vessel characteristics like gear and engine power or others. Our results show that vessel characteristics alone were not sufficient to describe fishing tactics, as there exist several tactics for a given gear and (or) a given set of vessel characteristics. Second, accounting for fishing tactics is useful for improving the evaluation of the impact of a fishery on the corresponding resources. Species-directed efforts and corresponding abundance indices may be calculated per tactic and utilized in current assessment models, e.g., for calibrating VPA. Finally, the tactics can serve to build a model of the dynamics of a mixed fishery. Such a model may be used to quantitatively assess the consequences of management measures on stock dynamics. Murawski and Finn (1986) and Marchal and Horwood (1996) developed models that provide the optimal fishing effort allocation between several fishing tactics. Laurec et al. (1991) used typologies of fishing trips in a simulation model of the Celtic Sea French groundfish fishery that aimed at evaluating the consequences of technological interactions. Laloë and Samba (1991) presented a model of the Senegal small-scale fishery that mimics the selection of fishing tactics on the basis of expected revenues. The typology of fishing tactics in the Celtic Sea fishery is currently used to develop a model of the fisheries dynamics that will serve to evaluate the consequences of different kinds of management measures.

In mixed fisheries, the exploitation simultaneously affects a number of stocks. Single-species management measures such as total allowable catch are not adequate for controlling exploitation levels (Murawski et al. 1983; Lewy and Vinther 1994) and can, for instance, lead to increased discarding for the less profitable species (Paulik et al. 1967). The importance of fishing location and seasonality in the definition of the tactics suggests that these should be explicitly taken into account in population dynamics models and stock assessment. In this perspective, identifying fishing tactics is only a starting point; it would be necessary to trace the activity of each fishing unit throughout the year. This falls beyond the scope of this paper and requires further data collection and analyses. Nevertheless, analyzing and modelling fishing activity with reference to fishing location and seasonality remains indispensable for the evaluation of alternative management measures like limiting fishing effort for either certain tactics or certain zones during some periods of the year.

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References

Bertignac, M. 1992. Les rendements par espèce de la pêche

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chalutière hauturière française de l'Atlantique Nord-Est entre 1972 et 1989. Aquat. Living Resour. **5**: 261–275.

Biseau, A., and Gondeaux, E. 1988. Apport des méthodes d'ordination en typologie des flottilles. J. Cons. Int. Explor. Mer, **44**: 286–296.

Centre International de Statistiques et d'Informatique Appliquées (CISIA). 1996. SPAD.N version 3. CISIA, Saint-Mandé, France.

Diday, E., Lechevallier, Y., and Opitz, O. 1996. Ordinal and symbolic data analysis. Springer-Verlag, Berlin.

He, X., Bigelow, K.A., and Boggs, C.H. 1997. Cluster analysis of longline sets and fishing strategies within the Hawaii-based fishery. Fish. Res. **31**: 147–158.

Hilborn, R., and Ledbetter, M. 1979. Analysis of the British Columbia salmon purse-seine fleet: dynamics of movement. Can. J. Fish. Aquat. Sci. **36**: 384–391.

Hilborn, R., and Ledbetter, M. 1985. Determinants of catching power in the British Columbia salmon purse seine fleet. Can. J. Fish. Aquat. Sci. **42**: 51–56.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment. Chapman and Hall, New York.

Laloë, F., and Samba, A. 1991. A simulation model of artisanal fisheries of Senegal. ICES Mar. Sci. Symp. **193**: 281–286.

Laurec, A., Biseau, A., and Charuau, A. 1991. Modelling technical interactions. ICES Mar. Sci. Symp. **193**: 225–236.

Lebart, L., Morineau, A., and Warwick, K.M. 1984. Multivariate descriptive statistical analysis. Correspondence analysis and related techniques for large matrices. John Wiley & Sons, New York.

Lewy, P., and Vinther, M. 1994. Identification of Danish North Sea trawl fisheries. ICES J. Mar. Sci. **51**: 263–272.

Marchal, P.J., and Horwood, J.W. 1996. Long-term targets for the Celtic Sea mixed-species multi-métiers fisheries. Aquat. Living Resour. **9**: 81–94.

McQueen, J.B. 1967. Some methods for classification and analysis of multivariate observations. Proc. Symp. Math. Stat. Prob. (5th). Vol. 1. University of California Press, Berkeley, Calif. pp. 281–297.

Murawski, S.A. 1984. Mixed-species yield per recruitment analyses accounting for technological interactions. Can. J. Fish. Aquat. Sci. **41**: 897–916.

Murawski, S.A., and Finn, J.T. 1986. Optimal effort allocation among competing mixed-species fisheries, subject to fishing mortality constraints. Can. J. Fish. Aquat. Sci. **43**: 90–100.

Murawski, S.A., Lange, A.M., Sissenwine, M.P., and Mayo, R.K. 1983. Definition and analysis of multispecies otter-trawl fisheries off the northeast coast of the United States. J. Cons. Int. Explor. Mer, **41**: 13–27.

Paulik, G.J, Hourston, A.S., and Larkin, P.A. 1967. Exploitation of multiple stocks by a common fishery. J. Fish. Res. Board Can. **24**: 2527–2537.

Rice, J. 1985. A brief guide to ordination methods. Can. Tech. Rep. Fish. Aquat. Sci. No. 1347. pp. 119–131.

Rogers, J.B., and Pikitch, E.K. 1992. Numerical definition of groundfish assemblages caught off the coasts of Oregon and Washington using commercial fishing strategies. Can. J. Fish. Aquat. Sci. **49**: 2648–2656.

Sampson, D.B. 1991. Fishing tactics and fish abundance, and their influence on catch rates. ICES J. Mar. Sci. **48**: 291–301.

Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. **58**: 236–244.

Appendix

Definition of the distance between individuals for PCA, TWCA, and MCA

For PCA, the distance between two individuals i and i' is the usual Euclidean distance:

$$\text{(A1)} \quad d^2_{\text{PCA}}(i,i') = \sum_{j=1}^{p} (x_{ij} - x_{i'j})^2.$$

When the variables exhibit very different ranges, too much weight may be given to variables with higher ranges of values than others. To avoid this, a normalized distance is used (see table below) to give identical weights to variables.

For TWCA and MCA, the distance between two individuals is called the χ^2 distance, i.e., a weighted Euclidean distance in which the weight of each category is the inverse of its frequency in the data set. Although TWCA is generally applied to a two-way contingency table, it may also be used (and will be used in this paper) for a data matrix X where the columns correspond to the p categories of a single variable and each row corresponds to an individual, x_{ij} being a positive measure of the level of the jth category for the ith individual. The distance between two individuals i and i' is defined as

$$\text{(A2)} \quad d^2_{\text{TWCA}}(i,i') = \sum_{j=1}^{p} \frac{x_{\bullet\bullet}}{x_{\bullet j}} \left(\frac{x_{ij}}{x_{i\bullet}} - \frac{x_{i'j}}{x_{i'\bullet}} \right)^2$$

where $x_{\bullet j} = \sum_{i=1}^{n} x_{ij}$, $x_{i\bullet} = \sum_{j=1}^{p} x_{ij}$, and $x_{\bullet\bullet} = \sum_{i=1}^{n} \sum_{j=1}^{p} x_{ij}$.

For MCA, the data matrix X is such that the columns correspond to the p categories of v qualitative variables, and $x_{ij} = 1$ when the ith individual exhibits the jth category (j ranging from 1 to the total number of categories over variables) and 0 otherwise. The distance between two individuals i and i' is defined as

$$\text{(A3)} \quad d^2_{\text{MCA}}(i,i') = \frac{1}{v} \sum_{j=1}^{p} \frac{n}{x_{\bullet j}} (x_{ij} - x_{i'j})^2$$

where $x_{\bullet j} = \Sigma_{i=1}^{n} x_{ij}$ is the number of individuals exhibiting the jth category.

Following eqs. A.2 and A.3, two individuals are close if they exhibit the same categories of the variable(s).

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Because the initial coordinates of the individuals correspond to values taken by one or several variables, the factorial analyses presented not only allow the study of the resemblances and differences between individuals, but also the analysis of the relationships between variables and the contribution of each variable to the variability of the data set. In normalized PCA, the similarity between any two variables is measured by the linear correlation coefficient. In MCA and TWCA, the distance between two categories is the χ^2 distance in which the weight of each individual is the inverse of its frequency in the data set.

Derivation of factorial axes

The spanning vectors of the factorial axes are the normalized eigenvectors of the square matrix of dimension p:

(A4) $A = {}^tZMZW$

where W is a diagonal matrix of weights for the p variables, M is a diagonal matrix of weights for the n individuals, and Z is closely linked to X. Consistent with the distances between individuals, the matrices Z, W, and M are defined as follows:

	PCA	TWCA	MCA
Z	$\begin{cases}\text{if normalized } z_{ij} = \dfrac{x_{ij} - \bar{x}_j}{s_j} \text{ with } s_j^2 = \displaystyle\sum_{i=1}^{n} m_i (x_{ij} - \bar{x}_j)^2 \\ \text{otherwise } z_{ij} = x_{ij} - \bar{x}_j\end{cases}$	$z_{ij} = \dfrac{x_{ij}}{x_{i\bullet}}$	$z_{ij} = \dfrac{x_{ij}}{v}$
W	$w_j = 1$	$w_j = \dfrac{x_{\bullet\bullet}}{x_{\bullet j}}$	$w_j = \dfrac{nv}{x_{\bullet j}}$
M	$m_i = \dfrac{1}{n}$	$m_i = \dfrac{x_{i\bullet}}{x_{\bullet\bullet}}$	$m_i = \dfrac{1}{n}$

Coordinates of individuals and variables

The coordinates F of the n individuals in the vector space spanned by the eigenvectors (termed "factorial coordinates") and the coordinates V of the p variables in that same vector space are

(A5) $\begin{cases} F = ZWU \\ V = WU\Lambda^{1/2} \end{cases}$

Equation A5 enables one to compute the factorial coordinates of any individual for which the same p variables have been measured.

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Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores

Amy D. Rosemond, Patrick J. Mulholland, and Susan H. Brawley

Abstract: We determined whether there were seasonal changes in the relative importance of consumers and resources in controlling stream periphyton. Our analysis included effects on algal populations and assemblage biomass and productivity. We used factorial experiments in which we manipulated snail densities, irradiance, and streamwater nutrient concentrations during two seasons, fall and spring, and compared responses with previously published summer findings. Periphyton biomass and productivity were much greater when snails were removed and nutrients and light were elevated during all seasons, indicating that all three factors were limiting or nearly limiting throughout the year. However, the relative importance of factors shifted seasonally. Irradiance limited periphyton biomass in summer and fall but not spring. In contrast, nutrients were more limiting in seasons in which light levels were higher: nutrient addition generally resulted in effects of greater magnitude in fall and spring than in summer. Snail growth was stimulated by enhanced irradiance in summer ($p = 0.06$) and by nutrient addition in fall, indicating resource limitation of both periphyton and snails. However, top-down control of periphyton by snails was also important: snails maintained low biomass assemblages dominated by only a few grazer-resistant species (e.g. basal cells of *Stigeoclonium tenue*, *Chamaesiphon investiens*) during all seasons.

Résumé : Nous avons examiné les variations saisonnières de l'importance relative des consommateurs et des ressources limitant le périphyton des cours d'eau. Notre analyse a traité des effets sur les populations d'algues et la biomasse et la productivité des communautés. Nous avons utilisé des plans d'expérience factoriels avec lesquels nous avons modifié la densité des escargots, l'éclairement et les concentrations de matières nutritives dans l'eau pendant deux saisons, l'automne et le printemps, et ensuite comparé les réactions à des résultats déjà publiés obtenus en été. La biomasse et la productivité du périphyton étaient de beaucoup supérieures en l'absence d'escargots et en présence de beaucoup de matières nutritives et de lumière cela pendant toutes les saisons, ce qui montre que ces trois facteurs étaient des facteurs limitants ou presque limitants pendant toute l'année. Par ailleurs, l'importance relative des facteurs se modifiait au cours des saisons. L'éclairement limitait la biomasse du périphyton en été et en automne mais non au printemps. Au contraire, les matières nutritives avaient un effet plus limitant au cours des saisons où l'éclairement était plus important : l'ajout de matières nutritives donnait généralement lieu à des effets de plus grande ampleur à l'automne et au printemps, comparativement à l'été. La croissance des escargots était stimulée par un plus important éclairement en été ($p = 0.06$) et par l'ajout de matières nutritives en automne, ce qui montre une limitation des ressources du périphyton et des escargots. Par ailleurs, la réduction verticale du périphyton par les escargots était aussi importante : les escargots maintenaient des communautés de faibles biomasses dominées par quelques espèces résistantes au broutage (p. ex. cellules basales de *Stigeoclonium tenue*, *Chamaesiphon investiens*) pendant toutes les saisons.

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Introduction

The balance between resource availability and consumption ultimately determines the biomass and production of trophic levels. "Bottom-up" effects of resources are predicted to be relatively more important at lower trophic levels, and "top-down" effects of consumers are predicted to be relatively more important at higher trophic levels (McQueen et al. 1986; Brett and Goldman 1997; but see Osenberg and Mittelbach 1994). Both factors have been found to have effects on at least one trophic level in most cases when resource and consumer effects have been tested simulta-

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neously (Brett and Goldman 1997). Less well studied are the effects of individual population contributions to changes in biomass and productivity of trophic levels. However, some studies have shown that the relative influence of resources and consumers may depend on the specific taxa that are present as producers and consumers (Leibold and Wilbur 1992; Osenberg and Mittelbach 1994; Rosemond and Brawley 1996).

The relative influence of resources and consumers on a given trophic level can also change seasonally (Vanni and Temte 1990). In eutrophic lakes, there can be large seasonal fluctuations in phytoplankton biomass (Reynolds 1984). Vanni and Temte (1990) found that changes in phytoplankton biomass were caused by different factors during different seasons. Grazing by zooplankton reduced phytoplankton in spring, but nutrient limitation was relatively more important than grazing in the summer. There may be less seasonal variation in oligotrophic systems, where resource limitation potentially has overriding control. In oligotrophic Lawrence Lake, nutrient addition and predator removal both affected populations of fish, invertebrates, and algae, but nutrient effects were greater than those of consumers for all three trophic levels (Osenberg and Mittlebach 1994). Likewise, in the Baltic Sea, where inorganic nutrient concentrations are low, nutrient limitation was more important than grazing effects on phytoplankton biomass and productivity during the growing season (Kivi et al. 1993). In Walker Branch (WB), where this study was conducted, there is little seasonal variability in periphyton biomass (Rosemond 1994). Previous work in this stream has shown that both low availability of resources (e.g., streamwater nutrients, irradiance) and high densities of consumers (snails) maintain periphyton biomass and productivity at low levels (Steinman 1992; Rosemond et al. 1993). Similarly, periphyton was limited by light, nutrients, and grazing snails in a stream near WB (Hill et al. 1992, 1995).

In this study, we determined seasonal changes in the relative influence of nutrients, light, and snail grazing on stream periphyton in WB. Our analyses included effects on assemblage biomass and productivity and also the underlying effects on algal populations that drive the biomass and productivity response. To test hypotheses concerning temporal changes in the relative importance of different limiting factors, we manipulated levels of each of these factors (light, nutrients, snail density) in stream-side channels during three different seasons (summer, fall, spring) to reduce their potential constraints on periphyton (e.g., light and nutrients were increased, snails were excluded). The results of the summer study have been published previously (Rosemond 1993). Here, we present the results of studies conducted in the fall and spring and also include data from the summer study in a new analysis of seasonally shifting limitation of stream periphyton.

Materials and methods

Study site

The West Fork of WB is located on the Department of Energy's Oak Ridge Reservation in Oak Ridge, Tenn. (35°58′N, 84°17′W). The stream flows through a deciduous forest watershed that has been undisturbed since ca.1940. Yellow poplar (*Liriodendron tulipifera* L.), beech (*Fagus grandifolia* Ehrh.), and oaks (*Quercus prinus* L., *Quercus alba* L.) dominate the riparian vegetation (Johnson and Van Hook 1989). The stream is primarily spring fed, with base flow accounting for 77% of the annual stream flow (Luxmoore and Huff 1989). Average stream depth is approximately 5–10 cm and average width is approximately 1.5–3 m at base flow (Mulholland 1992).

Nutrient concentrations in WB are relatively low during all seasons ($\leq 50\ \mu g$ inorganic $N \cdot L^{-1}$, $\leq 7\ \mu g\ P \cdot L^{-1}$) but are higher in the summer months than at other times of the year (Mulholland 1992). There is also distinct seasonality in irradiance levels, which are low in the summer months, increase following litterfall, and exhibit an annual peak in early March and April (Rosemond 1994). The most abundant herbivore in WB is the snail *Elimia clavaeformis* (family Pleuroceridae), which occurs at densities >1000·m^{-2} year-round (Rosemond 1994) and comprises >95% of the invertebrate biomass in the stream (Newbold et al. 1983). Weekly water temperature readings (range) during each of the experiments indicated slightly higher temperatures during summer (16°C) than during fall (9–14°C) or spring (10.5–14°C). Based on seasonal trends in potentially limiting factors, we hypothesized that light would be least limiting in the spring, when levels were highest, and nutrients would be least limiting in the summer, when their concentrations are highest. Since snails occur at consistently high densities, we hypothesized that they would constrain periphyton response to seasonal changes in resource availability.

Experimental

Two experiments were conducted in flow-through channels for approximately 7 weeks, each with approximately 2 months between experiments: (*i*) a fall study from 3 November 1989 to 20 December 1989 and (*ii*) a spring study from 7 March 1990 to 26 April 1990. The previously reported summer study was conducted from 21 July 1989 to 8 September 1989. The channels, described in Rosemond (1993), were placed next to the West Fork of WB, from which water for the channels was pumped.

The effects of light (high or ambient), nutrients (high or ambient), and snail herbivory (absent or ambient) were tested using a complete factorial design. This design resulted in eight different treatment combinations, which were duplicated and randomly assigned among 16 stream-side channels. Unglazed ceramic tiles (upper surface area = 5.3 cm^2), which had been placed in WB at least 6 weeks prior to each study, were used as substrates for algal colonization in the channels. They were transferred to the channels approximately 2–3 weeks prior to the start of each experiment.

Snail densities used in the experiments were determined from bimonthly measurements in WB using a 250-cm^2 underwater viewer (n = 40 for each measurement). Grazing treatments consisted of (*i*) snails added at ambient densities (approximately 1100·m^{-2}) and (*ii*) no snails. Ambient light conditions were measured monthly on cloudless days using ozalid paper light meters (Friend 1961) along a 150-m reach of stream. Light was increased in all experiments by suspending two lamps with 1000-W metal halide bulbs (Sylvania Metalarc) over the channels. Ambient light treatments were then shaded with layers of mosquito netting to simulate natural light levels. Lamps were placed on a 10-h photoperiod for both experiments.

Nutrient concentrations were increased to approximately 10 times yearly average stream concentrations by continuous addition of concentrated nutrient solutions to the high-nutrient channels (using Mariotte bottles: Peterson et al. 1983). Nutrient treatments consisted of (*i*) ambient (no addition) and (*ii*) addition of both inorganic N and P to $210\ \mu g\ N \cdot L^{-1}$ as $NaNO_3$, $40\ \mu g\ N \cdot L^{-1}$ as NH_4Cl, and $35\ \mu g\ P \cdot L^{-1}$ as K_2HPO_4 (in fall, but H_3PO_4 in spring). Water samples were taken weekly from the channels and filtered in the field through precombusted, washed glassfiber filters (Gelman type A/E, pore size = 1 μm) for nutrient analyses. Concentrations of

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Table 1. Stream water nutrient concentrations ($\mu g \cdot L^{-1}$) in low- (ambient) and high-nutrient treatments in the fall (6 November to 11 December 1989) and spring (8 March to 24 April 1990) experiments.

	Ambient channels			High-nutrient channels		
	SRP	NO_3	NH_4	SRP	NO_3	NH_4
Fall						
Mean	1.8	12.0	6.3	41.2	223.0	51.9
Range	1.1–2.5	5.4–18.4	0.8–16.3	28.7–54.1	105.0–411.5	35.4–84.4
Spring						
Mean	2.4	10.1	2.0	49.8	270.2	51.0
Range	1.7–4.0	6.8–13.5	0–5.0	38.0–72.0	233.3–365.3	17.0–83.3

Note: Mean concentration is based on six weekly averages in fall and seven weekly averages in spring with ranges. Samples for weekly averages from nutrient-enriched stream-side channels ($n = 8$) and from ambient stream water ($n = 8$ for fall, $n = 1$ for spring) were taken at approximately the same time.

soluble reactive P (SRP) were measured using the ascorbic acid method (American Public Health Association 1985), ammonium N (NH_4^+-N) by phenate colorimetry using an autoanalyzer (Technicon TRAACS 800), and nitrate plus nitrite N ($(NO_3^- + NO_2^-)$-N) by Cu–Cd reduction followed by automated colorimetric analysis (United States Environmental Protection Agency 1983). Carbon fixation rates were determined from an average of five tiles from each channel at the beginning and end of each experiment. Tiles were collected from the channels and placed in recirculating chambers containing 1 L of filtered (Gelman type A/E filter) water from the corresponding channel. Light levels during each incubation were maintained similar to treatment levels. $NaH^{14}CO_3$ (specific activity 0.74 $MBq \cdot mmol^{-1}$) was added to the water in each chamber and ^{14}C uptake during a 3-h incubation was determined, following extraction of cell contents in dimethyl sulfoxide and assay of ^{14}C by liquid scintillation on subsamples of the extract (Palumbo et al. 1987). Carbon fixation rates were normalized by tile area to yield area-specific productivity (ASP).

Ash-free dry mass (AFDM) of periphyton was estimated at the beginning and end of each study by collecting five tiles from each channel, placing the tiles with attached periphyton in aluminum pans, and determining differences between dry (at 60°C for 24 h) and ash (at 500°C for 24 h) masses.

Five additional tiles were collected from each channel at the beginning and end of each study to determine whether there were changes in algal community structure in response to experimental treatments. Algae were brushed from the tiles and preserved in 2% glutaraldehyde. Samples were processed with a tissue homogenizer, as required, to break up large aggregations and then sonicated. Algal units (single cells or colonies, >300 per sample) were counted and identified at 400× using a Palmer-Maloney cell. Diatoms were lumped into groups or genera when counted at 400×. Identifications of diatoms to species were then made at 1000× from permanent slides and the groups or genera initially counted were further subdivided. For each taxon, length, width, and depth or diameter of spherical cells (1–20 per taxon) were measured with an ocular micrometer and biovolume was estimated using geometric formulae modified from Kellar et al. (1980).

As an indicator of P limitation, alkaline phosphatase activity (APA) was determined on an average of five tiles from each channel at the end of each experiment. *p*-Nitrophenyl phosphate (NPP) (Sigma) was added to small jars containing filtered stream water and a tile with attached periphyton. Following a 30-min incubation, the stream water was filtered (Whatman GFF glassfiber filters) and absorbance of the filtrate was determined spectrophotometrically to determine hydrolysis of NPP (after the pH was raised to ≥10 by adding 0.05 mL of 1 N NaOH). In our previous analysis (Rosemond 1993), we had normalized APA per unit of chlorophyll *a*. However, because chlorophyll *a* content per cell likely changed with light treatment and season, we expressed APA here on an areal basis. APA was calculated as the amount of NPP hydrolyzed (moles of nitrophenol produced) per tile area.

Snail growth was determined as the average AFDM gain of snails from each channel during the experiment. Averages were based on $n = 85$ per channel in fall (total = 680) and $n = 20$ per channel in spring (total = 160). Individual snail widths were measured with calipers to the nearest 0.01 mm and measured snails were marked with paint and (or) bee tags (Chr. Graze, K.G., Weinstadt, Germany). Widths of each marked snail were remeasured at the end of each experiment. Increases in AFDM over the experimental periods (final average minus initial average) were determined using a width–AFDM regression computed from snails previously taken from WB ($AFDM = 1.2332 \times 10^{-5}(width)^{3.98}$, $n = 50$, $R^2 = 0.96$). Growth was expressed as percent AFDM increase over the experimental period (47 days for fall, 50 days for spring).

Statistical analyses

Data were analyzed by a three-factor (nutrients, light, grazing) analysis of variance (ANOVA) using the ANOVA procedure of Statistical Analysis Systems (SAS Institute, Inc. 1985). Values used in the analyses for AFDM, ASP, and APA were means from five tiles from each replicate channel ($n = 2$ per treatment). The model that we used tested for main effects of grazing (G), light (L), and nutrients (NU) and all possible interactive effects (G × L, G × NU, L × NU, and G × L × NU). Algal biovolumes were log transformed prior to analyses (Zar 1984). Treatment effects on biovolumes of dominant species (comprising >10% biovolume in more than nine samples) were also tested by ANOVA. Species of *Achnanthes* (*A. minutissima*, *A. lanceolata*, *A. lanceolata* var. *dubia*, *A. exigua*) were grouped for statistical tests. Snail growth data were analyzed using a two-factor (L, NU) ANOVA. For all data, if treatment effects were significant by ANOVA, post-ANOVA multiple comparison tests were conducted for each season using Ryan's *Q* test (Day and Quinn 1989). AFDM and ASP were measured before treatments were started, and ANOVAs on these data indicated no differences among treatment groups prior to the experiments.

Results

Nutrient concentrations, light levels, and snail densities

Nutrient concentrations were increased to roughly the same levels during both experiments, representing a 10- to 20-fold increase over ambient levels (Table 1). Ambient concentrations were very similar in fall and spring, and these were slightly lower than summer levels (Rosemond 1993). Irradiance levels in the stream and in ambient channels were lower in fall than in spring (Table 2). Experimental increases in irradiance were additive to ambient light, resulting in higher irradiance in "high-light" channels in spring than in

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Table 2. Light levels (photosynthetically active radiation, mmol quanta·m^{-2}·day^{-1}) in the stream and in stream-side channels.

Date	Stream	Ambient channels	High-light channels
Fall			
9 Oct. 1989	517 (62) (n = 12)		
18 Nov. 1989		490 (n = 1)	7334 (n = 1)
30 Nov. 1989	1251 (70) (n = 10)	1216 (n = 1)	8356 (2358) (n = 2)
26 Jan. 1990	1199 (51) (n = 12)		
Spring			
4 Mar. 1990	4234 (90) (n = 12)		
4 Apr. 1990	4267 (92) (n = 11)	2700[a]	11 830 (558) (n = 3)
24 Apr. 1990	2121 (96) (n = 12)		
30 May 1990	526 (81) (n = 11)		

Note: Data are means (SE) (number of samples).
[a]Estimated from instantaneous readings.

Table 3. Treatment effects on periphyton biomass as AFDM and ASP.

Treatment	df	AFDM	ASP
Fall			
G	1	(–) 94.80****	(–) 72.24****
L	1	(+) 7.23*	(+) 11.22**
NU	1	(+) 48.85****	(+) 77.14****
G × L	1	21.16**	21.01**
G × NU	1	32.88***	47.56****
L × NU	1	6.60*	24.70***
G × L × NU	1	12.42***	31.27***
Spring			
G	1	(–) 13.77**	(–) 150.62****
L	1	ns	(+) 23.40**
NU	1	(+) 9.96**	(+) 152.59****
G × L	1	ns	15.16**
G × NU	1	8.13*	72.78****
L × NU	1	ns	20.99**
G × L × NU	1	ns	11.22**

Note: Data are F values from ANOVA of week 7 measurements. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$; ns, not significant. Treatment effects: G, grazing; L, light; NU, nutrients. + and – indicate the direction of the treatment effect.

fall. Ambient light levels were maintained at a constant level during the spring experiment, when light levels in the stream were fairly constant. During the fall experiment, two light levels were used, low levels initially (similar to summertime levels) and then higher levels approximately 3 weeks after the start of the experiment (starting on 28 November 1989), to simulate the ambient increases in light following leaf fall.

Snail densities in the stream were fairly constant over the study period (Rosemond 1994), and experimental densities used in grazed channels were similar to those in the stream (1042 snails·m^{-2} in fall and 1103 snails·m^{-2} in spring). Densities of snails observed foraging on tile surfaces were somewhat lower than stocking densities (snails not on tiles were on channel walls or in crevices between tiles). The mean of counts on tile surfaces made during the fall was 354 snails·m^{-2} (range 256–561, n = 7), approximately one-third of the densities initially stocked. These counts were not made during the spring study, but conditions were similar to those in the fall. Any observed snail immigrants were removed from ungrazed channels every 1–2 days. Low densities of mayflies (primarily *Baetis* spp.) colonized channels but were also removed every 1–2 days when present.

Effects on periphyton biomass and productivity

Snail grazing had negative effects and nutrient addition had positive effects on periphyton AFDM in both the fall and spring (Table 3; Fig. 1). In addition, significant G × NU interactions were likely the result of greater effects of nutrients when grazers were absent. Enhanced light had positive effects on AFDM in the fall but not in the spring (Table 3). All treatment effects and interaction terms on ASP in both seasons were significant (Table 3), with the highest ASP observed in ungrazed/+NU&L treatments (Fig. 2).

Effects on index of P limitation

Nutrient additions had a negative effect on APA during spring and fall (Fig. 3; Table 4). Although APA was also generally lower with nutrient addition in summer, high variability resulted in no significant effect of nutrient addition. APA was also reduced by grazing in fall but not during the other two seasons (Table 4).

Effects on algal populations

In general, those species that persisted in the presence of grazers were not those that responded to increases in resources when grazers were removed. Grazed communities were composed largely of basal cells of *Stigeoclonium tenue* (Kuetz.) (Chlorophyta) and *Chamaesiphon investiens* (Cyanophyta) in spring and fall (Figs. 4*a* and 5*a*). Grazing had positive effects on *Chamaesiphon* biovolume, and increased irradiance had no effect (during spring) or negative effects (during fall) on both *Chamaesiphon* and *Stigeoclonium* biovolumes (Table 5). In contrast, species that dominated communities when grazers were removed increased in biovolume in response to increased resources. Ungrazed communities were composed of several diatom species (predominately *Melosira varians* (Ag.), *Meridion circulare* (Grev.), *Cocconeis placentula* (Ehr.), *Achnanthes* (Bory) spp., and *Eunotia pectinalis*) in both spring and fall (Figs. 4*b* and 5*b*). Grazing had negative effects and increased resources (light and nutrients) had positive effects on the dominant diatom taxa (Table 5). These species responded positively to increased irradiance in fall but less so in spring (Table 5).

Effects of nutrients and light on snail growth

Nutrient addition had significant positive effects on snail growth in the fall (results of two-way ANOVA: light effect not significant, nutrient effect significant at $p < 0.05$). Snail growth rates were generally higher in +NU&L treatments in both seasons (Fig. 6). No statistically significant effects of nutrient addition or enhanced light were observed in the

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Fig. 1. Periphyton AFDM in different treatments during the last week of the (*a*) fall experiment (3 November to 20 December 1989) and (*b*) spring experiment (7 March to 26 April 1990). Ambient: shaded, low-nutrient treatments; +L, high-light, low-nutrient treatments; +NU, shaded, high-nutrient treatments; +NU&L, high-light, high-nutrient treatments. Solid bars, grazed (snails added at ambient densities); open bars, ungrazed (no snails added). Lines above bars represent ±1 SE. Means of bars with the same letter are not significantly different by post-ANOVA multiple comparison tests. Note that the AFDM values in Rosemond (1993) are an order of magnitude lower than they should be due to a calculation error.

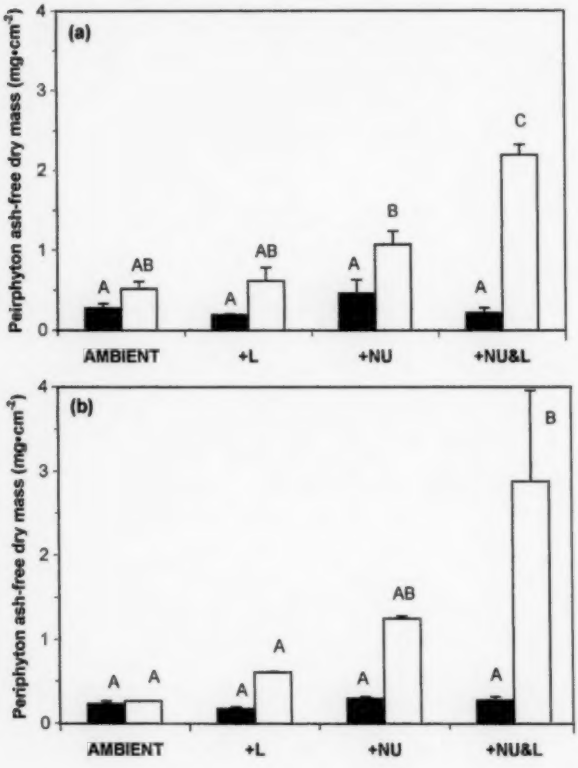
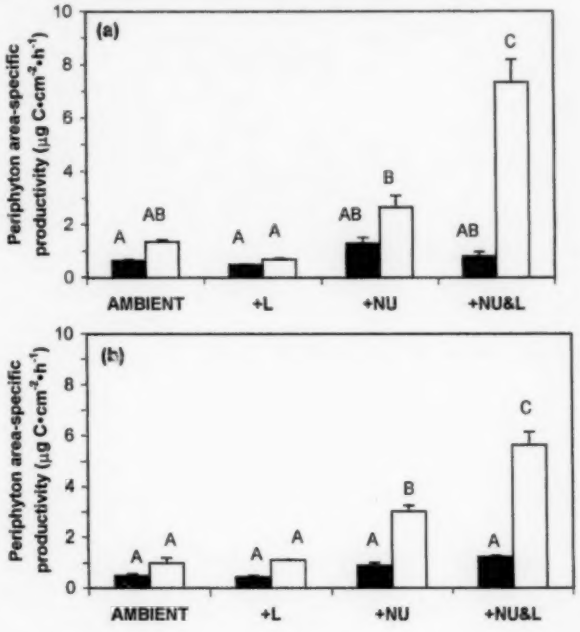


Fig. 2. Area-specific periphyton productivity in the different treatments during the last week of the (*a*) fall experiment and (*b*) spring experiment. Treatments and symbols as in Fig. 1.



spring. Comparison of the magnitude of daily growth rates (μg/day (SD)) by season indicates the highest growth rates and greatest overall response to resource addition in summer. Daily growth rates in ambient, +L, +NU, and +NU&L treatments in fall were 2.87 (0.15), 1.91 (0.90), 3.72 (0.75), and 4.79 (0.75); in spring, 1.90 (2.12), 7.70 (0.71), 7.20 (3.68), and 10.40 (4.52); and in summer, 7.04 (3.61), 13.67 (1.15), 10.92 (6.78), and 17.55 (1.15). In all seasons, the highest snail growth rates were observed in treatments in which both irradiance and nutrient concentrations were elevated.

Discussion

Differences in the effects of grazing, light, and nutrients among seasons

During both fall and spring, periphyton biomass and productivity were considerably greater when snails were removed and light and nutrients were elevated together than when any one or two of these factors were manipulated. Similar results were obtained in the summer study reported earlier (Rosemond 1993). This suggests that all three of these factors were at or near limiting levels year-round. These results contrast with those of Vanni and Temte (1990), indicating a clear shift from limitation due to consumers in spring to limitation due to resources in summer. In WB, both consumers and resources appear to be important constraints on periphyton, regardless of season.

Although all three factors were at or near limiting levels during different seasons, their relative importance as constraints on periphyton changed somewhat with season. This appears to be a result of changes in the prevailing nutrient and light conditions. There were positive effects of light on periphyton biomass and biomass of several algal populations in the fall but not in the spring when irradiance was already sufficiently high. The effects of enhanced light, however, appeared to be greatest in summer, when increased irradiance resulted in the greatest increase in periphyton biomass (in ungrazed/+L treatments, compared with other seasons) and enhanced light increased snail growth (Rosemond 1993).

The response of periphyton biomass and productivity to nutrient addition was greater in seasons when light levels were greater. There were significant positive effects of nutrient addition during all seasons, but the magnitude of the effect of nutrients on biomass and productivity (in ungrazed treatments) was greater in fall and spring than in summer (compare with Rosemond 1993). Significant positive effects of nutrient addition on snail growth were only observed in

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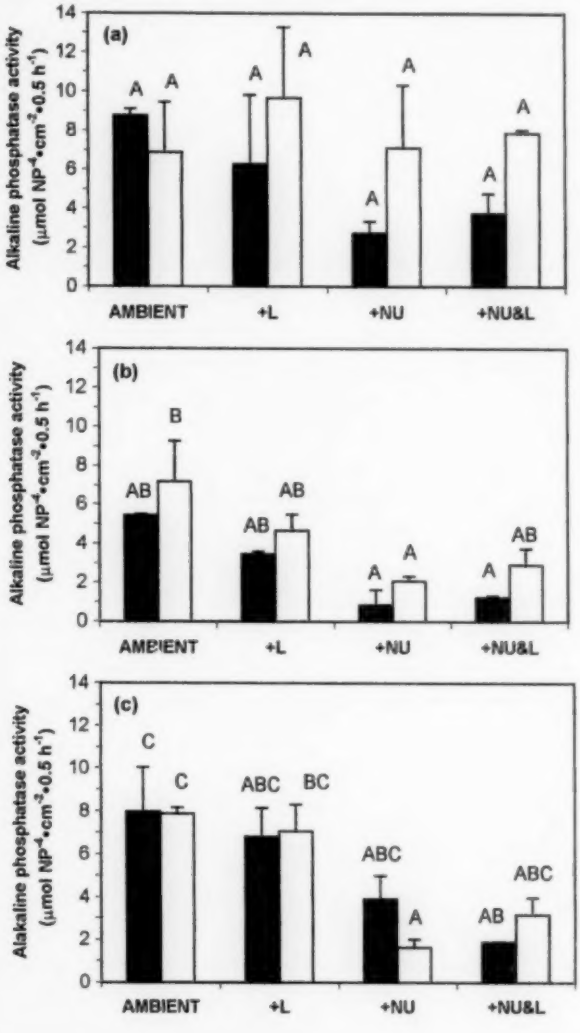
Fig. 3. APA in different treatments during the last week of the (*a*) summer experiment, (*b*) fall experiment, and (*c*) spring experiment. Treatments and symbols as in Fig. 1.



the fall; however, the magnitude of the effect of nutrient addition (compared to ambient conditions) on daily growth rates was actually greater in spring than in fall (mean difference of 5.3 μg/day in spring and 0.85 μg/day in fall between treatments). Other studies have shown that the effects of nutrient addition on stream periphyton are dependent on prevailing light conditions and that effects of irradiance are dependent on ambient nutrient concentrations (see references cited in Rosemond 1993). The results of our study further demonstrate that effects of nutrients and light on algal communities can be highly interdependent. Seasonal increases in light or nutrients may only have positive effects on algal communities that are not constrained by the other factor.

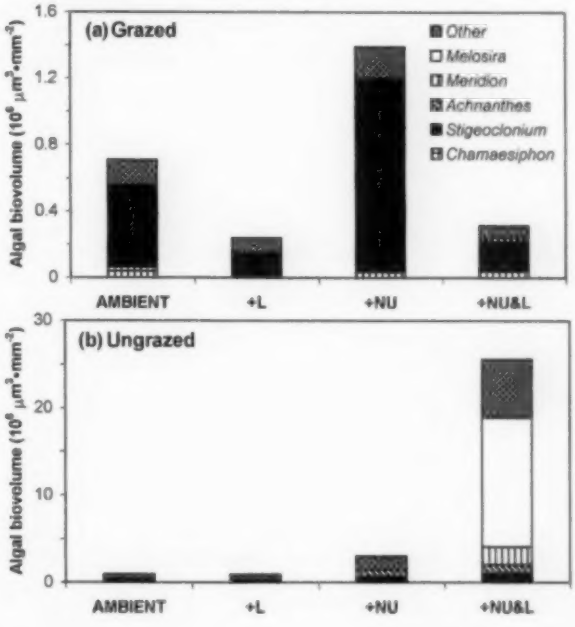
. In contrast with the effects of light and nutrients, which changed with season, snails appeared to have very similar

Table 4. Results of ANOVA on APA from summer, fall, and spring experiments.

Treatment	df	Summer	Fall	Spring
G	1	ns	(–) 5.70*	ns
L	1	ns	ns	ns
NU	1	ns	(–) 28.67***	(–) 38.08***
G × L	1	ns	ns	ns
G × NU	1	ns	ns	ns
L × NU	1	ns	4.70†	ns
G × L × NU	1	ns	ns	ns

Note: Here, we present results of APA normalized per area, in contrast with the results from the summer study previously reported in Rosemond (1993), where values were normalized by chlorophyll *a*. †$p \leq 0.10$, *$p \leq 0.05$, ***$p \leq 0.001$; ns, not significant. Treatment effects: G, grazing; L, light; NU, nutrients. – indicates the direction of the treatment effect.

Fig. 4. Algal species composition of (*a*) grazed and (*b*) ungrazed treatments at the end of the fall experiment based on biovolumes of *Chamaesiphon investiens*, *Stigeoclonium tenue*, *Achnanthes* spp., *Meridion circulare*, *Melosira varians*, and all other algal species. Note the different axes for the grazed and ungrazed treatments. Treatments and symbols as in Fig. 1. Note that *M. circulare* was misidentified as *Peronia intermedium* in two previous publications: Rosemond (1993) and Rosemond et al. (1993).



effects on periphyton, regardless of season. In this study, increases in nutrients and light were ultimately manifested in increased snail biomass, indicating that snails were able to harvest increases in algal productivity and maintain low algal biomass even when resources were increased. These experimental results corroborate field observations that biomass and productivity of periphyton changed very little seasonally, despite large seasonal changes in light levels and nutrient concentrations (Rosemond 1994).

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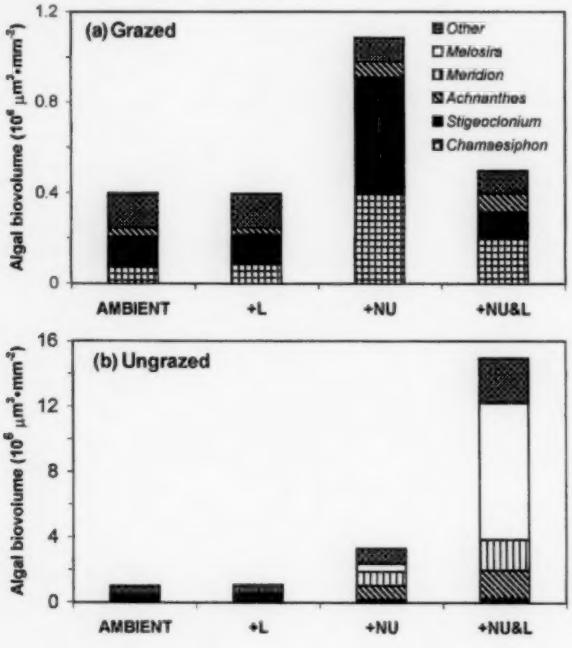
Fig. 5. Algal species composition of (*a*) grazed and (*b*) ungrazed treatments at the end of the spring experiment based on biovolume. Species designations, treatments, and symbols as in Fig. 4. Note the different axes for the grazed and ungrazed treatments.



Different algal species were abundant under grazed conditions regardless of nutrient and light levels (e.g., *Chamaesiphon* and *Stigeoclonium*) than when resources were increased and herbivores were removed (e.g., *Melosira* and *Meridion*). These results partially contrast with predictions of Osenberg and Mittelbach (1994) that the species composition of plants and prey should change across productivity gradients. In our study, algal species composition was largely constant over gradients of light and nutrients: algal populations were controlled more by the presence or absence of herbivores than by changes in resources. The changes in algal species composition that we observed are consistent with community structure being determined by "trade-offs" between herbivore resistance and competitive abilities, as has been suggested for other plant communities (Olsen and Lubchenco 1989; Huntly 1991). Manifestations of this trade-off (e.g., grazer-resistant plant species being overgrown by faster-growing species when herbivores were removed) have been observed previously in streams (Power et al. 1988; Rosemond et al. 1993), coral reefs (Hay 1981; Lewis 1986), intertidal communities (Lubchenco 1978; Steneck 1982), and terrestrial plant communities (Huntly 1987). Thus, algal populations that had high growth potential, such as the dominant diatom taxa observed in ungrazed treatments in this study, could respond to increases in resources, but only when grazers were excluded. WB may be a system that is chronically at the upper end of a spectrum of grazing intensity. In this study, and other studies in WB, herbivores were most important in determining community structure and largely constrained periphyton response to increased resources. However, in other systems where grazing pressure is lower, and as evidenced to some extent in ungrazed treatments in this study, species composition may change in response to gradients in resources (e.g., Osenberg and Mittelbach 1994).

Changing effects of stream herbivores under different resource conditions

Whereas snails had pronounced effects on species composition under ambient resource conditions, there was very little increase in biomass or productivity of periphyton when snails were removed without additional amendments of light or nutrients. In contrast, differences in biomass and productivity between grazed and ungrazed treatments in which light and nutrients were increased were great. Similarly, when both herbivores and growth-limiting resources have been manipulated in other streams, grazer effects were greatest at high resource levels (Feminella and Hawkins 1995).

These results differ from those of other studies that have found reduced effects of grazers under high resource conditions (e.g., Hansson 1992). In Hansson's (1992) study, grazers became less effective as lake water nutrient concentrations increased. In contrast, our findings appear to be a result of (*i*) herbivores being able to maintain low plant biomass under high resource conditions coupled with (*ii*) an increase in plant productivity in response to increased resource levels when herbivores were removed. Thus, the conditions that determine whether herbivores have reduced or increased effects on plant communities when resources are elevated may depend on whether or not herbivores are food limited and (or) plants are resource limited. Food limitation of consumers (e.g., Hill et al. 1995) and resource limitation of plants (e.g., Pringle 1987) may be common in streams. These conditions may result in relatively minor effects of herbivores on the biomass and productivity of plant communities under ambient conditions but greater effects when growth-limiting resources become abundant (e.g., storm-influenced nutrient pulses, seasonal changes in light availability).

What limits primary producers in streams? An analysis of proximate constraint

These studies illustrate that limitation is a relative term. Here, and in Rosemond (1993), any of the factors tested could only be considered to be primarily limiting when growth constraints due to other factors were removed. For example, for AFDM, nutrient addition had no effect with grazers present under ambient light conditions in the summer, but had a large effect when grazers were removed and light levels were elevated. Likewise, removal of grazers in the spring had no appreciable effect on periphyton biomass, but had substantial effects when nutrients were simultaneously increased. Interestingly, not one of these factors would have been identified as "limiting," had their effects been tested singly, as indicated by multiple comparison tests for differences in means for single-factor manipulations (e.g., grazed/ambient versus ungrazed/ambient or grazed/ambient versus grazed/+NU), despite the fact that 4- to 10-fold increases over ambient levels for most parameters were observed when all three factors were manipulated.

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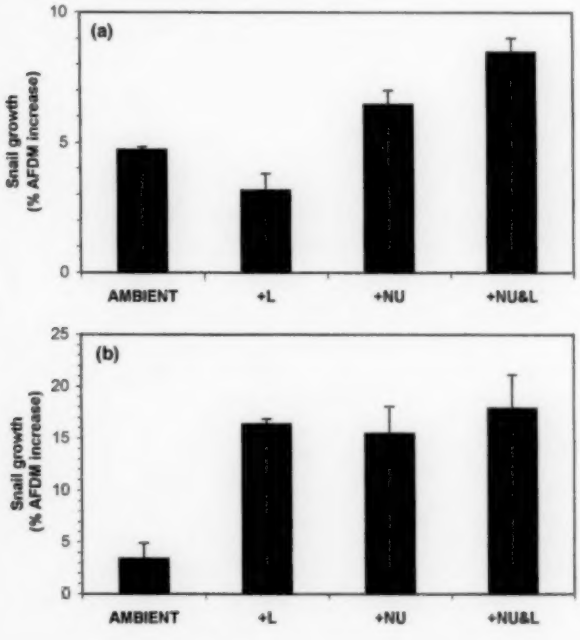
Table 5. Treatment effects on the biovolume of different algal species.

Treatment	df	CHAM	STIG	ACH	MERI	MELO
Fall						
G	1	(+) 11.04**	ns	(–) 314.17****	(–) 27.22***	(–) 16.72**
L	1	(–) 4.81†	(–) 10.99**	(+) 12.01**	(+) 4.98†	(+) 4.37†
NU	1	ns	(+) 14.93**	(+) 36.08***	ns	(+) 4.37†
G × L	1	ns	4.74†	11.91**	ns	4.37†
G × NU	1	ns	ns	49.74****	ns	4.37†
L × NU	1	ns	ns	8.35*	ns	16.72**
G × L × NU	1	4.25†	ns	3.59†	ns	16.72**
Spring						
G	1	(+) 7.06*	ns	(–) 58.74****	(–) 6454.49****	(–) 10.21**
L	1	ns	ns	ns	(+) 5.82*	ns
NU	1	ns	ns	(+) 31.11***	(+) 64.99****	(+) 3.89†
G × L	1	ns	ns	ns	5.82*	ns
G × NU	1	ns	ns	7.84*	64.99****	3.89†
L × NU	1	ns	ns	ns	ns	ns
G × L × NU	1	ns	ns	ns	ns	ns

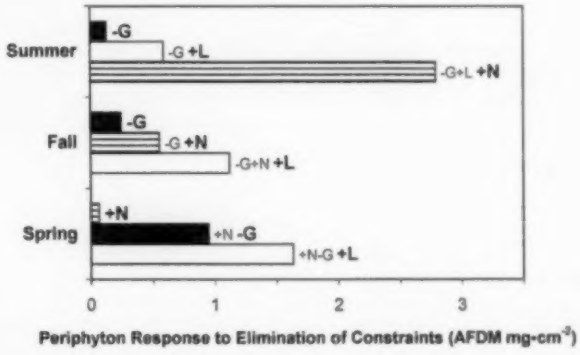
Note: Data are F values from ANOVA of log biovolume of *Chamaesiphon investiens* (CHAM) (division Cyanophyta), *Stigeoclonium tenue* (STIG) (division Chlorophyta), *Achnanthes* spp. (ACH), *Meridion circulare* (MERI), and *Melosira varians* (MELO). *Achnanthes* spp., *Meridion*, and *Melosira* are all diatoms (division Chrysophyta, class Bacillariophyceae). †$p \le 0.10$, *$p \le 0.05$,**$p \le 0.01$, ***$p \le 0.001$, ****$p \le 0.0001$; ns, not significant. Treatment effects: S, season; G, grazing; L, light; NU, nutrients. + and – indicate the direction of the treatment effect.

Fig. 6. Snail growth as percent increase over the (*a*) fall and (*b*) spring experimental periods. Treatments as in Fig. 1.



Periphyton biomass in WB appears to be constrained by colimitation of several factors. Nonetheless, each of these factors is not of equal importance. By examining the relative response to single- and combined-factor manipulations, we determined the order of importance of these factors as constraints on periphyton. In this way, we assessed whether the proximate limitation of periphyton biomass in this stream during different seasons was the high abundance of snails or

Fig. 7. Analysis of order (top to bottom within each season) of proximate limitation by nutrients (pinstriped bars), light (open bars), and grazing (solid bars) on periphyton AFDM. The length of each bar represents the increase in AFDM due to the removal of constraints due to each particular factor. Factors listed second or third for each season include effects of factors listed above them. See text for further explanation.



the low levels of light or nutrients. To do this, we determined which single constraint, when removed, resulted in the greatest increase in biomass or productivity under ambient levels of the other two factors. This analysis was conducted for periphyton AFDM and the results are depicted in Fig. 7. Each factor was assigned a position (top to bottom for each season) based on its proximate control of AFDM. The top factor is that which was closest to being the single most important factor in controlling AFDM during that season (it yielded the greatest increase in AFDM when its level was manipulated alone). The second factor in each vertical set yielded the greatest increase after the first factor had been increased (or removed, in the case of snails). For example, in the fall, removal of snails resulted in greater increases

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in AFDM over ambient, grazed levels (+0.24 mg·cm^{-2}) than an increase in nutrients (+0.18 mg·cm^{-2}) or an increase in light (–0.08 mg·cm^{-2}). Then, considering ungrazed treatments, increases in nutrients (+0.56 mg·cm^{-2}) resulted in greater increases in AFDM than increases in light (+0.10 mg·cm^{-2}). Finally, increasing light resulted in an AFDM increase of 1.12 mg·cm^{-2} under elevated nutrient conditions in the absence of grazing. Note that although increases in light after snails were removed and nutrients were increased resulted in the greatest periphyton AFDM response, light was the least important proximate limiting factor during the fall. The patterns of proximate limitation differed among seasons; factors were important in the order of G, L, and NU in the summer, G, NU, and L in the fall, and NU, G, and L in the spring. The same analysis was conducted for total algal biovolume for which similar results were obtained (factors were important in the order of G, L, and NU in the summer, NU, G, and L in the fall, and NU, G, and L in the spring).

The results of the preceding analyses help to illustrate the manner in which multiple constraints control periphyton in WB during different seasons. The initial hurdle to an increase in biomass in the summer and fall is to overcome losses due to snail grazing. Light was secondarily limiting during the summer, whereas nutrients were secondarily limiting during the fall. Nutrients were initially the most important constraint in the spring, but once supplied at sufficient concentrations, snail grazing limited biomass accrual. Nutrients were the least important constraint in the summer (when concentrations in stream water were highest), and light was the least important constraint during the fall and spring (when ambient light levels were highest), consistent with our a priori hypotheses.

A somewhat surprising result was that removal of grazers generally had a greater proximate effect on algal biomass than resource addition, although the ultimate effects of grazers in constraining periphyton response to resources had been predicted. Why were proximate effects of grazers so great? They were probably due to the identity of both the producers and consumer. Previous studies have shown that at least one of the grazer-resistant taxa in this study (*S. tenue*) has a lower biomass-specific productivity rate than an assemblage of diatom taxa, similar to the assemblages that were observed in ungrazed treatments in these studies (Rosemond and Brawley 1996). When grazers were removed, taxa with higher inherent rates of productivity replaced slow producers. In addition, the identity of the herbivore in this system is also clearly important. Many previous studies have shown negative effects of snails, including *E. clavaeformis*, on the biomass of stream algae (Feminella and Hawkins 1995).

Results of this study indicate that interactions between resources and consumers constrained biomass and productivity of stream periphyton. But are resources and consumers both important in controlling periphyton in other streams as well? Hydrologic disturbance, which was not considered in this study, can be a particularly powerful structuring force in streams. Many studies have found that hydrologic factors correlate with seasonal patterns of lotic primary producers (Wehr 1981; Duncan and Blinn 1989; Júnior et al. 1991). Other studies have found variation in resources to be most important in determining seasonal patterns in stream periphyton (Sumner and Fisher 1979; Keithan and Lowe 1985; Sheath and Burkholder 1985). Hydrologic disturbances, common in many streams, can also interact with resources or consumers to determine seasonal patterns. For example, Power (1992) has shown that the algae in some northern California rivers shift from being under hydrologic control during the winter wet season to consumer control during the dry season. Grimm and Fisher (1986) suggested that resource (nutrient) availability is important in algal regrowth following depression of biomass due to flood disturbance. Our study was conducted in a spring-fed stream, where disturbance due to flooding may be less important than in more hydrologically variable streams. Our results indicate that both consumption and resource limitation were important constraints on periphyton year-round and agree with predictions that biotic interactions can be important structuring factors in relatively benign environments such as spring-fed streams (Peckarsky 1983).

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References

American Public Health Association. 1985. Standard methods for the examination of water and waste water. 12th ed. American Public Health Association, Washington, D.C.

Brett, M.T., and Goldman, C.R. 1997. Consumer versus resource control in freshwater pelagic food webs. Science (Washington, D.C.), **275**: 384–386.

Day, R.W., and Quinn, G.P. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. **59**: 433–463.

Duncan, S.W., and Blinn, D.W. 1989. Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream. J. Phycol. **25**: 455–461.

Feminella, J.W., and Hawkins, C.H. 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. J. North Am. Benthol. Soc. **14**: 465–509.

Friend, D.T.C. 1961. A simple method of measuring integrated light values in the field. Ecology, **42**: 577–580.

© 2000 NRC Canada

Grimm, N.B., and Fisher, S.G. 1986. Nitrogen limitation in a Sonoran desert stream. J. North Am. Benthol. Soc. **5**: 2–15.

Hansson, L.-A. 1992. The role of food chain composition and nutrient availability in shaping algal biomass development. Ecology, **73**: 241–247.

Hay, M.E. 1981. The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. Ecology, **62**: 739–750.

Hill, W.R., Boston, H.L., and Steinman, A.D. 1992. Grazers and nutrients simultaneously limit lotic primary productivity. Can. J. Fish. Aquat. Sci. **49**: 504–512.

Hill, W.R., Ryon, M.G., and Schilling, E.M. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology, **76**: 1297–1309.

Huntly, N. 1987. Effects of refuging consumers (pikas: *Ochotona princeps*) on subalpine vegetation. Ecology, **68**: 274–283.

Huntly, N. 1991. Herbivores and the dynamics of communities and ecosystems. Annu. Rev. Ecol. Syst. **22**: 477–503.

Johnson, D.W., and Van Hook, R.I. (*Editors*). 1989. Analysis of biogeochemical cycling processes in Walker Branch watershed. Springer-Verlag, New York.

Júnior, O.N., Dip, M.R., and Góes, R.J. 1991. Macroalgae of a stream in southeastern Brazil: composition, seasonal variation and relation to physical and chemical variables. Hydrobiologia, **213**: 241–250.

Keithan, E.D., and Lowe, R.L. 1985. Primary productivity and spatial structure of phytolithic growth in streams in the Great Smoky Mountains National Park, Tennessee. Hydrobiologia, **123**: 59–67.

Kellar, P.E., Paulson, S.A., and Paulson, L.J. 1980. Methods for biological, chemical, and physical analyses in reservoirs. Lake Mead Limnological Research Center, Tech. Rep. No 5.

Kivi, K., Kaitala, S., Kuosa, H. Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B., and Tamminen, T. 1993. Nutrient limitation and grazing control of the Baltic phytoplankton community during annual succession. Limnol. Oceanogr. **38**: 893–905.

Leibold, M.A., and Wilbur, H.M. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature (Lond.), **360**: 341–343.

Lewis, S.M. 1986. The role of herbivorous fishes in the organization of a Caribbean reef community. Ecol. Monogr. **56**: 183–200.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. Am. Nat. **112**: 23–39.

Luxmoore, R.J., and Huff, D.D. 1989. Water. *In* Analysis of biogeochemical cycling processes in Walker Branch watershed. *Edited by* D.W. Johnson and R.I. Van Hook. Spinger-Verlag, New York. pp. 164–196.

McQueen, D.J., Post, J.R., and Mills, E.L. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. **43**: 1571–1581.

Mulholland, P.J. 1992. Regulation of nutrient concentrations in a temperate forest stream: roles of upland riparian and instream processes. Limnol. Oceanogr. **37**: 1512–1526.

Newbold, J.D., Elwood, J.W., O'Neill, R.V., and Sheldon, A.L. 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. Ecology, **64**: 1249–1265.

Olsen, A.M., and Lubchenco, J. 1989. Competition in seaweeds: linking plant traits to competitive outcomes. J. Phycol. **26**: 1–6.

Osenberg, C.W., and Mittelbach, G.G. 1994. The relative importance of resource limitation and predator limitation in food chains. *In* Food webs: integration of patterns and dynamics. *Edited by* G.A. Polis and K.O. Winemiller. Chapman and Hall, New York. pp. 134–148.

Palumbo, A.V., Mulholland, P.J., and Elwood, J.W. 1987. Extraction with DMSO to simultaneously measure periphyton photosynthesis, chlorophyll, and ATP. Limnol. Oceanogr. **32**: 464–471.

Peckarsky, B.L. 1983. Biotic interactions or abiotic limitations? A model of lotic community structure. *In* Dynamics of lotic ecosystems. *Edited by* T.D. Fontaine III and S.M. Bartell. Ann Arbor Science Publishers, Ann Arbor, Mich. pp. 303–323.

Peterson, B.J., Hobbie, J.E., Corliss, T.L., and Kriet, K. 1983. A continuous-flow periphyton bioassay: tests of nutrient limitation in a tundra stream. Limnol. Oceanogr. **28**: 583–591.

Power, M.E. 1992. Hydrologic and trophic controls of seasonal algal blooms in northern California rivers. Arch. Hydrobiol. **125**: 385–410.

Power, M.E., Stewart, A.J., and Matthews, W.J. 1988. Grazer control of algae in an Ozark mountain stream: effect of short-term exclusion. Ecology, **69**: 1894–1898.

Pringle, C.M. 1987. Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. Can. J. Fish. Aquat. Sci. **44**: 619–629.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, U.K.

Rosemond, A.D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia, **94**: 585–594.

Rosemond, A.D. 1994. Multiple factors limit seasonal variation in periphyton in a forest stream. J. North Am. Benthol. Soc. **13**: 333–344.

Rosemond, A.D., and S.H. Brawley. 1996. Species-specific characteristics explain the persistence of *Stigeoclonium tenue* (Chlorophyta) in a woodland stream. J. Phycol. **32**: 54–63.

Rosemond, A.D., Mulholland, P.J., and Elwood, J.W. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology, **74**: 1264–1280.

SAS Institute, Inc. 1985. SAS user's guide: basics. Version 5. SAS Institute, Inc., Cary, N.C.

Sheath, R.G., and Burkholder, J.M. 1985. Characteristics of softwater streams in Rhode Island. II. Composition and seasonal dynamics of macroalgal communities. Hydrobiologia, **128**: 109–118.

Steinman, A.D. 1992. Does an increase in irradiance influence periphyton in a heavily-grazed woodland stream? Oecologia, **91**: 163–170.

Steneck, R.S. 1982. A limpet – coralline algal association: adaptations and defenses between a selective herbivore and its prey. Ecology, **63**: 507–522.

Sumner, W.T., and Fisher, S.G. 1979. Periphyton production in Fort River, Massachusetts. Freshwater Biol. **9**: 205–212.

United States Environmental Protection Agency. 1983. Methods of chemical analysis of water and wastes. No. EPA-600/4-79-020. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. Rep.

Vanni, M.J., and Temte, J. 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. Limnol. Oceanogr. **35**: 697–709.

Wehr, J.D. 1981. Analysis of seasonal succession of attached algae in a mountain stream, the North Alouette River, British Columbia. Can. J. Bot. **59**: 1465–1474.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.

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Factors influencing invertebrate communities in prairie wetlands: a multivariate approach

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Abstract: We examined the relationships between invertebrate community structure and a number of biotic and abiotic variables in 19 semipermanent prairie wetlands. We tested whether aquatic invertebrate communities differed (*i*) between wetlands with and without fathead minnows (*Pimephales promelas*) and (*ii*) according to drainage history of wetlands (restored versus natural, nondrained). We also evaluated influences of other environmental variables on invertebrate community structure, including abundance of aquatic macrophytes and amphibians and wetland depth and surface area. Invertebrate communities differed significantly between wetlands with and without fathead minnows, largely due to lower relative abundance of 19 invertebrate taxa (of 32 taxa analyzed) in wetlands with fathead minnows. In contrast, we found no differences in these taxa between natural and restored wetlands. Canonical correspondence analysis indicated that invertebrate community structure was affected by abundance of fathead minnows, abundance of aquatic macrophytes, and wetland depth, with fathead minnows the most influential variable measured. Many studies have documented the effects of fish predation on zooplankton communities, but our results show that fathead minnows in prairie wetlands affect a large number of diverse invertebrate taxa. The presence of these fish results in an invertebrate community distinctly different from that found in fishless wetlands.

Résumé : Nous avons étudié les relations entre la structure des communautés d'invertébrés et un certain nombre de variables biotiques et abiotiques dans 19 milieux humides semi-permanents des prairies. Nous avons vérifié si les communautés d'invertébrés aquatiques diffèrent (*i*) entre les milieux humides où se trouvent des têtes-de-boule (*Pimephales promelas*) et où il n'y en a pas et (*ii*) selon l'historique du drainage des milieux humides (s'ils sont restaurés ou naturels et non drainés). Nous avons aussi étudié l'influence d'autres variables environnementales sur la structure des communautés d'invertébrés, comme l'abondance de macrophytes aquatiques et d'amphibiens et la profondeur et l'étendue du milieu humide. Les communautés d'invertébrés diffèrent énormément entre les milieux humides avec ou sans têtes-de-boule, ce qui est, en grande partie, le résultat de la faible abondance relative de 19 taxons d'invertébrés (sur les 32 analysés) dans les milieux humides où vivent des têtes-de-boule. Par ailleurs, nous n'avons dénoté aucune différence pour ces taxons entre les milieux humides naturels et restaurés. L'analyse de correspondance canonique a indiqué que la structure des communautés d'invertébrés était influencée par l'abondance de têtes-de-boule et de macrophytes aquatiques et par la profondeur du milieu humide, l'abondance de têtes-de-boule étant la variable mesurée la plus déterminante. Plusieurs études ont documenté les effets de la prédation des poissons sur les communautés de zooplancton, mais nos résultats montrent que la présence des têtes-de-boule dans les milieux humides des prairies affecte un grand nombre de différents taxons d'invertébrés. La présence de ces poissons produit une communauté d'invertébrés totalement différente de celle que l'on retrouve dans les milieux humides dont ils sont absents.

[Traduit par la Rédaction]

Introduction

Aquatic invertebrates play an important role in wetland food webs, serving as a crucial link between primary production and secondary consumers (Murkin and Wrubleski 1988). In the Prairie Pothole Region (PPR), aquatic invertebrates are an important food resource for nesting female ducks and prefledgling ducklings, as well as adult and larval tiger salamanders (*Ambystoma tigrinum*) (Bouffard and Hanson 1997). Invertebrates are also an important food resource for terrestrial species more peripherally associated with wetland habitats, such as shorebirds, passerines, and other birds (Euliss et al. 1999). It is likely that decreased invertebrate abundance may lead to lower production rates for species that depend on invertebrates as a food resource. For example, Cox et al. (1998) showed that growth and survival of mallard ducklings (*Anas platyrhynchos*) were directly related to total abundance of aquatic invertebrates. Extensive loss of wetlands increases the value of ecological functions performed by remaining sites, including production of aquatic invertebrates (Bellrose and Low 1978). Thus, identifying variables that influence invertebrate abundance and community structure is a high research and management priority.

Many factors structure aquatic invertebrate communities. These include agricultural chemicals (Dieter et al. 1996), land management practices (Szalay and Resh 1997), hydrology and water chemistry (Euliss et al. 1991), hydroperiod (Neckles et al. 1990), nutrient enrichment (Pettigrew et al.

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1998), and presence of fish such as fathead minnows (*Pimephales promelas*) (Hanson and Riggs 1995). Influences of fish on invertebrate communities in wetlands may be particularly important, given the effects of fish documented in other aquatic habitats such as rivers (Power 1990) and lakes (Carpenter and Kitchell 1993). In the PPR, fathead minnows are probably the fish species with the greatest potential to influence invertebrate communities. These fish are native to wetlands having a permanent or semipermanent water regime (sensu Stewart and Kantrud 1971), existing as metapopulations in which a proportion of populations in individual wetlands is eliminated by winter hypoxia. Thus, high spatial variability and temporal variability are typical features of these populations. Because fathead minnows are commercially reared as bait fish in semipermanent wetlands throughout the midwestern United States, stocking may stabilize natural density fluctuations and increase the proportion of wetlands supporting populations. Commercial rearing occurs on a large scale; Litvak and Mandrak (1993) reported sales of cultured and wild-harvested bait fish from nine states to exceed $367 million in 1991. Integration of watersheds by installation of ditches, culverts, and drainage tile likely increases the overall dispersion of these fish and increases the probability of colonization of additional wetlands.

Fathead minnows may alter the abundance, production, and community structure of aquatic invertebrates. These fish feed primarily on aquatic invertebrates (Price et al. 1991), and populations are capable of explosive growth (Payer and Scalet 1978). Duffy (1998) estimated that consumption rates of fathead minnows may roughly equal annual production rates of aquatic invertebrates in semipermanent wetlands, and Hanson and Riggs (1995) showed that the abundance of vulnerable invertebrate taxa was reduced by these fish in wetlands. Through indirect effects, some taxa may respond positively to the presence of fish.

Invertebrate community structure reflects the influence of many variables, and discrete influences are difficult to identify. We used multivariate techniques of classification and ordination to examine relationships among aquatic invertebrate community structure and seven biotic and abiotic variables. Differences in hydroperiod, salinity, and surrounding land use have been identified as important determinants of aquatic invertebrate communities in prairie wetlands. The wetlands that we examined were similar in these factors, and we focused on variables that have not been thoroughly studied, yet vary considerably among wetlands of the PPR. Specifically, we examined the influences of time (year), wetland history (restored versus natural, nondrained), average wetland depth, surface area, and abundance of fathead minnows, aquatic macrophytes, and amphibians on the structure of aquatic invertebrate communities in prairie wetlands.

Materials and methods

Study sites

The wetlands that we examined were semipermanent (following the classification of Stewart and Kantrud 1971), drying approximately once every 10 years. Study wetlands were selected from a population of candidate wetlands on private and federal lands within the PPR of west-central Minnesota. The restored wetlands used in our study were previously drained by ditching and had been restored 5–10 years earlier by installation of earthen dikes and water control structures. Ten restored wetlands were selected for study based on criteria including presence or absence of fathead minnows, upland cover type, similarities in hydroperiod, specific conductance, and other physical characteristics. Five restored wetlands had fathead minnow populations and five were fishless, based on the results of sampling during spring 1996. We also selected 10 natural wetlands (nondrained) based on similar criteria, five with and five without fathead minnow populations. Our intention was that study sites consist of semipermanent wetlands as similar as possible for all unmeasured variables in order to minimize confounding influences.

Field sampling methods

Sampling was conducted from May through August in 1996 and 1997. Five random transects were established in each wetland in both years, and all sampling was done along these transects. Surface area of each wetland was obtained from data provided by U.S. Fish and Wildlife Service offices in Detroit Lakes and Fergus Falls, Minnesota. Average depth was determined from measurements that we recorded at 16 locations along the transects.

Abundance of fathead minnows was indexed at the beginning (early May), middle (July), and end (August) of each field season with funnel-type minnow traps. Five traps were set at the interface of open water and emergent macrophytes in each wetland and collected after 24 h (Hanson and Riggs 1995). Results for each date were expressed as the total biomass of each fish species captured in the five traps. The average total biomass of fathead minnows captured per date was calculated from the three sampling dates for each wetland, and this seasonal mean was used for statistical analyses. Fish species other than fathead minnows were occasionally captured, mainly brook sticklebacks (*Culaea inconstans*) and central mudminnows (*Umbra limi*). However, fathead minnows were overwhelmingly dominant in wetlands with fish, with other species representing less than 0.1% of fish biomass captured in both years. Given the predominance of fathead minnows, only fathead minnow data were used in our analyses.

Abundance of amphibians was measured in May, July, and August each year with the same traps that we used to sample fish. We captured both adult and larval frogs and tiger salamanders. Results for each date were expressed as the total number of amphibians captured in the five traps, and a seasonal mean was calculated for each wetland from the total number captured on the three sampling dates. As with fish, these seasonal means were used for subsequent analyses.

Aquatic invertebrates were sampled with integrated column samplers (Swanson 1978) and activity traps (ATs) following the design of Murkin et al. (1983). Each year, samples were collected every third week from May through August. Column samples were taken at five random locations in the open water of each wetland on each date and concentrated by filtration through a 68-μm-mesh funnel. Ten ATs were deployed in each wetland on each date, one at the emergent vegetation – open water interface and one in open water along each of the five transects. Traps were suspended 30 cm below the water surface and retrieved after 24 h. AT contents were concentrated by passing samples through a 68-μm-mesh funnel. Both column and AT samples were preserved with 70% ethanol.

Invertebrates were identified to the lowest feasible taxonomic group and counted. Level of taxonomic resolution varied, but identification was made to suborder, family, or genus level. The total number of invertebrates in each taxonomic group captured with the five column samples and 10 ATs on each date for each wetland was determined. The seasonal average for each taxonomic group in each wetland was calculated from 90 samples over six sampling dates and used in our analyses.

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Abundance of aquatic macrophytes was assessed using a weighted plant rake (Depp and Lathrop 1992) in August each year. In each wetland, 15 sampling stations were randomly selected and four rake-throws were taken at each station. The number of times that an individual species was collected was used as an index of each species' abundance (60 possible throws). Counts for all species were summed to provide a measure of total macrophyte abundance in each wetland, and these sums were used in the statistical analyses.

Statistical analysis

All analyses were performed using PC-ORD for Windows (McCune and Mefford 1997). We performed an initial analysis for statistical outliers using our invertebrate data and calculated the average Euclidean distance among wetland scores. Wetlands with scores greater than 2.0 standard deviations from the mean were excluded from further analyses. Results indicated that one wetland was a significant outlier in both 1996 and 1997, and this wetland was excluded from further analyses. This site was the only wetland in which a small number of black bullheads (*Ameiurus melas*) were captured. Thus, we used 19 wetlands for the remainder of our analyses. Environmental data were relativized by the maximum to standardize for different measurement scales, and invertebrate data were transformed to $\ln(n + 1)$ prior to analysis to prevent high values from excessively influencing our results (ter Braak 1995).

We used cluster analysis to group together wetlands with similar invertebrate communities. Clusters of wetlands were then compared with respect to a priori wetland dichotomies (restored or natural, presence or absence of fish, 1996 or 1997) to determine if strong relationships existed (van Tongeren 1995). Our cluster analysis was performed using Euclidean distance and the group-linkage method of flexible beta (with beta equal to –0.25; Ludwig and Reynolds 1988).

Unfortunately, cluster analysis provides no information regarding the nature of differences between clusters of wetlands. Thus, we used the indicator test of Dufrene and Legendre (1997) to characterize faunal differences between wetland clusters. We also applied this test to each of the a priori wetland dichotomies. This test compares the relative frequency of occurrence and abundance of taxa in different groups of sites and identifies taxa that vary more between wetland groups than would be expected by chance. We tested the significance of each invertebrate taxon as an indicator with Monte Carlo tests (1000 permutations) (Dufrene and Legendre 1997).

Cluster analysis separates and groups relatively homogenous communities, making statistical significance of clusters difficult to determine (Ludwig and Reynolds 1988). We tested for significant differences in invertebrate communities at the two-cluster level and between a priori wetland dichotomies using multiresponse permutation procedures (MRPP) (Biondini et al. 1988). This is a data-dependent permutation test based on Euclidean distances that can be performed on a number of groups and requires no assumptions about the distribution of the data.

We used direct (canonical correspondence analysis (CCA)) and indirect (principal components analysis (PCA)) ordination techniques to examine relationships among invertebrate community structure and the continuous environmental variables of wetland depth, surface area, and abundance of fathead minnows, aquatic macrophytes, and amphibians. Both techniques were used because of strengths and shortcomings associated with each method when describing community structure and relating it to environmental variables. With CCA, the relationship between environmental variables and community structure is "directly" assessed, and the relationships can be tested statistically using Monte Carlo tests. However, CCA results can be misleading if noisy or irrelevant environmental data are included in the analysis and site scores are plotted as linear combinations of environmental variables (LC scores) (ter Braak 1995; McCune 1997). Additionally, because site scores in CCA are constrained by environmental variables, true community structure and gradients may not be expressed, and McCune (1997) concluded that CCA LC scores should not be used to describe community structure. In contrast, indirect ordination with PCA accurately portrays community structure and gradients because environmental data are not used in the ordination (ter Braak 1995), thereby avoiding some of the potential pitfalls of CCA. With indirect ordination, environmental data are subsequently analyzed with the ordination results to determine if relationships exist between community and environmental gradients. The shortcoming of indirect ordination in PCA is that the relationships between invertebrate communities and environmental data are not assessed directly, and the significance of relationships cannot be tested statistically. Similar results with PCA and CCA are mutually supportive and indicate that important environmental variables have been included in the analysis (ter Braak 1995).

We performed PCA on the invertebrate data, and following the suggestions of ter Braak (1995), our analysis was species centered (variance–covariance for the cross-products matrix) but not standardized. We developed a joint plot with environmental variables and invertebrate taxa, allowing assessment of relationships among ordination results and specific taxa and environmental variables (ter Braak 1995). Only environmental variables and invertebrate taxa with $r^2 > 0.20$ for either the first or second axis were portrayed. Statistical significance of axes generated with PCA was inferred using Fisher's (1958) ratio test.

We used forward selection to determine which environmental variables were significantly related to the invertebrate data prior to CCA (ter Braak and Verdonschot 1995). The P values of both marginal and conditional effects were adjusted with a sequential Bonnferoni correction (Rice 1990). Only variables with significant marginal and conditional effects were included in CCA. For CCA, rows and columns were standardized by centering and normalizing. Following the suggestions of Palmer (1993), diagrams were constructed with site scores as linear combinations of variables (LC scores). Statistical significance of eigenvalues and species–environment correlations for the first three axes generated in CCA were tested with Monte Carlo tests based on 1000 permutations (ter Braak and Verdonschot 1995).

Results

Cluster analysis separated wetlands into two well-defined groups, wetlands with fathead minnows and fishless sites (Fig. 1). In contrast, there was no apparent difference between restored and natural wetlands, as these two types of wetlands clustered randomly within the fish and fishless groups. Invertebrate communities varied considerably within a wetland from year to year, as relatively few individual wetlands clustered together both years. However, all wetlands remained in either the fish or fishless group both years. Indicator tests showed 20 taxa (of 32 analyzed) to be significant at the two-cluster level, which represents fish-containing versus fishless wetlands (Fig. 1). Nineteen of the 20 taxa had higher relative abundances in fishless wetlands, with only Corixidae more abundant in wetlands with fathead minnows (Fig. 1). As all 20 significant indicator taxa occurred in both fish-containing and fishless wetlands, the difference in invertebrate community structure between these two wetland groups results from differences in the abundance of taxa shown in Fig. 1. No taxa were significant indicators for the a priori dichotomies of restored versus natural wetlands or wetlands sampled in 1996 versus 1997, supporting the results of our cluster analysis.

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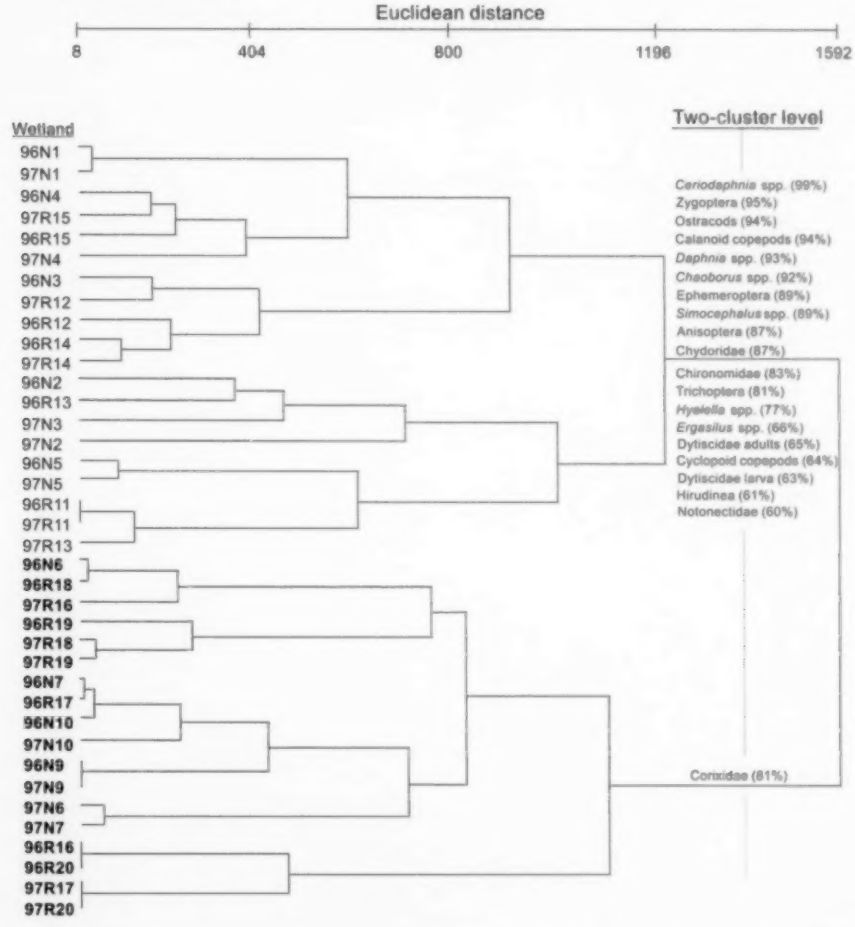
Table 1. Results of MRPP tests for significant differences in invertebrate communities between the a priori dichotomies of wetlands with fathead minnows versus fishless wetlands in 1996 and 1997, restored versus natural wetlands in 1996 and 1997, and wetlands sampled in 1996 versus 1997.

	1996		1997	
Test	*R*	*P*	*R*	*P*
Fish versus no-fish wetlands	0.182	0.000006	0.178	0.000001
Restored versus natural wetlands	–0.016	0.88	–0.008	0.59
		R	*P*	
1996 versus 1997 wetlands		0.007	0.16	

Note: *R* is the chance-corrected within-group similarity where $R_{max} = 1$ when all invertebrate communities are identical within groups, $R = 0$ when heterogeneity of communities within groups equals expectation by chance, and $R < 0$ when heterogeneity of communities within groups exceeds that expected by chance.

Fig. 1. Results of cluster analysis on invertebrate communities in 19 wetlands sampled in 1996 and 1997. The first two numbers in each wetland's designation indicate year, R indicates restored wetlands, N indicates natural wetlands, and the numbers to the right of the letters indicate specific wetlands. Wetlands in boldface type contain fathead minnows, and those in lightface type are fishless. Note that the two-cluster level distinguishes wetlands with fathead minnows from fishless sites. Significant indicator taxa ($P < 0.05$) at the two-cluster level are listed with the cluster of wetlands where their relative abundance was higher, with the percentage of total individuals captured in that cluster in parentheses.



MRPP tests between wetlands with and without fathead minnows were significant in both 1996 and 1997, indicating differences in invertebrate communities between these two types of wetlands (Table 1). Additionally, *R* values (within-group similarity) show less heterogeneity of communities within these groups of wetlands than would be expected by

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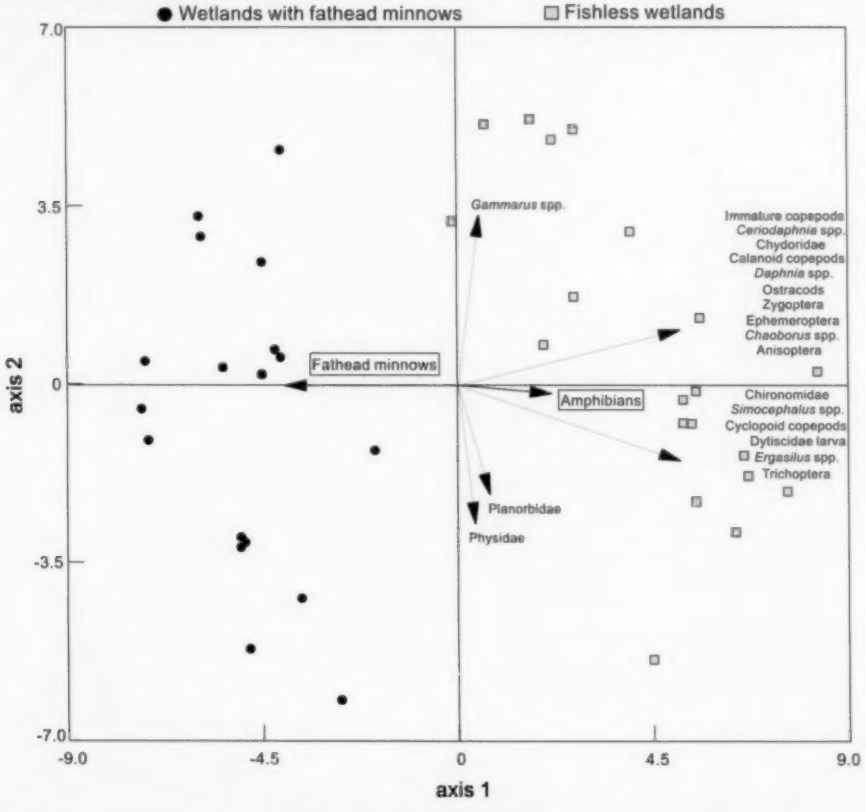
Table 2. Marginal and conditional effects of environmental variables determined by forward selection in CCA.

	Marginal effects		Conditional effects		
Environmental variable	Eigenvalue	*P*	Eigenvalue	*P*	Cumulative eigenvalue
Fathead minnows*	0.032	0.004[a]	0.032	0.002[a]	0.032
Aquatic plants*	0.023	0.004[a]	0.020	0.002[a]	0.052
Average depth*	0.021	0.004[a]	0.013	0.002[a]	0.065
Surface area	0.016	0.090			
Amphibians	0.014	0.130			

Note: Environmental variables significant for both effects were included in subsequent CCA and are indicated by an asterisk. Statistical significance was determined using Monte Carlo tests (1000 permutations).

[a]*P* value adjusted with sequential Bonnferoni correction (Rice 1990).

Fig. 2. Results of PCA on invertebrate communities and joint plot with those environmental variables (solid arrows) and invertebrate taxa (broken arrows) having $r^2 > 0.20$ for either axis. Vectors (arrows) point in direction of increasing values for the respective variables. Longer vectors indicate stronger correlations between wetland scores and the vector variables. Not all vectors are shown for invertebrate taxa on the right-hand side of the figure, but all vectors for these taxa fell between the two vectors shown. *Gammarus* spp., Physidae, and Planorbidae were correlated with axis 2 and all other invertebrate taxa, amphibians, and fathead minnows with axis 1. Data are from 19 wetlands sampled in 1996 and 1997.



chance. No significant difference was detected between restored and natural wetlands in either year or between 1996 and 1997 wetlands, and *R* values indicated that heterogeneity of communities within these wetland groups equals or exceeds that expected by chance (Table 1).

PCA of invertebrate communities revealed two groups representing wetlands with and without fathead minnows (Fig. 2). Fishless wetlands had positive scores on axis 1 (with one marginal exception), whereas wetlands with fathead minnows had negative scores on that axis. Our joint plot shows abundances of fathead minnows and amphibians to be the only environmental variables correlated with either of the first two PCA axes, with fathead minnows negatively related to axis 1 ($r^2 = 0.45$) and amphibians positively related to the same axis ($r^2 = 0.28$). Sixteen invertebrate taxa were positively correlated with axis 1. Negative relationships between abundances of these invertebrates and fathead minnows are indicated by the opposite directions of their re-

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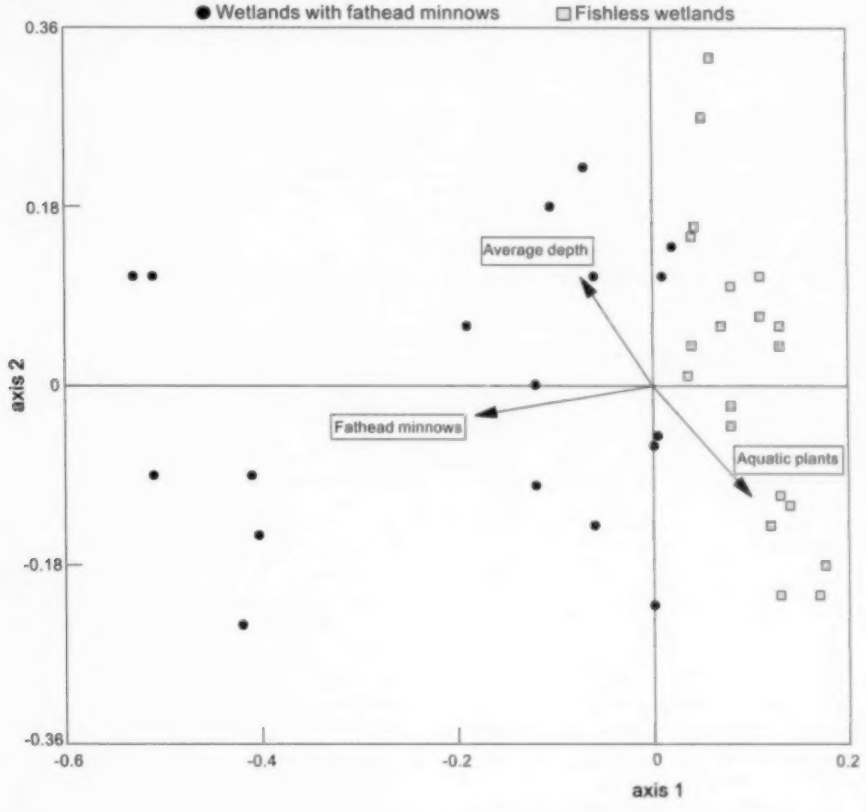
Fig. 3. Results of CCA on invertebrate communities and environmental variables in 19 wetlands sampled in 1996 and 1997. Vectors (arrows) point in the direction of increasing values for the respective variables, with longer vectors indicating stronger correlations between vectors and axes. Coefficient of determination (r^2) for fathead minnows and axis 1 is 0.97, for aquatic plants and axis 2 is 0.70, and for average depth and axis 2 is 0.76.



spective vectors. The average correlation coefficient between these taxa and axis 1 was 0.45, with a maximum of 0.90 for *Ceriodaphnia* spp. Three taxa were correlated with axis 2, *Gammarus* spp. in a positive relationship ($r^2 = 0.53$) and Planorbidae ($r^2 = 0.29$) and Physidae ($r^2 = 0.32$) in a negative relationship. The first five PCA axes each explained a significant amount of variance in our invertebrate data compared with a random model (all $P < 0.05$), with axes 1 and 2 explaining 40 and 14% of the total variance, respectively.

We performed CCA using only fathead minnows, aquatic plants, and average depth, as these were the only environmental variables with significant marginal and conditional effects (Table 2). Forward selection indicated the most influential environmental variable to be fathead minnow abundance, followed by abundance of aquatic plants and depth. CCA results were quite similar to the results of PCA performed on the invertebrate data in terms of wetland scores and the length and direction of the fathead minnow vector (Fig. 3). Direction and strength of environmental vectors indicate a negative relationship between fathead minnows and plant abundance and a much stronger negative relationship between average depth and plant abundance. Overall, eigenvalues were relatively small, but results of Monte Carlo tests indicated that values for the first three axes were significant (Table 3). Correlations between species and environmental data were moderate, and Monte Carlo tests again indicated that all three axes were statistically significant (Table 3). Intraset correlations and coefficients of determination show that axis 1 is a gradient of decreasing fathead minnow abundance ($r^2 = 0.97$) and that axis 2 is a gradient of increasing average depth ($r^2 = 0.76$) and decreasing plant abundance ($r^2 = 0.70$) (Table 4). None of the environmental variables showed a strong relationship with axis 3.

Ordination scores for invertebrate taxa indicate a negative relationship between a number of taxa and fathead minnows and a positive relationship between other taxa and aquatic plants (Fig. 4). Taxa to the right along axis 1 are negatively related to the abundance of fathead minnows, and this relationship is strongest for *Daphnia* spp., Anisoptera, *Chaoborus* spp., *Simocephalus* spp., and *Ceriodaphnia* spp. Hydrophilidae and Haliplidae larvae have the strongest positive relationship with the abundance of aquatic plants and negative relationship with depth; Hydracarina and immature copepods show the opposite relationships. Corixidae show the strongest positive association with fathead minnows. In general, wetlands without fathead minnows had higher abundances of taxa on the right-hand side of axis 1.

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Table 3. Results of CCA on invertebrate communities and environmental data in 19 wetlands sampled in 1996 and 1997.

Axis summary statistics	Axis 1	Axis 2	Axis 3
Eigenvalue	0.034	0.020	0.013
% of variance explained	11.5	7.0	4.3
Pearson correlation between species and environment	0.83	0.72	0.65
	P		
Significance tests	Axis 1	Axis 2	Axis 3
Eigenvalue	0.001	0.001	0.001
Pearson correlation between species and environment	0.001	0.004	0.010

Note: Shown are the results for the first three axes and the significance tests for the eigenvalues and species–environment correlations shown in the axis summary statistics. Significance was determined with Monte Carlo tests (1000 permutations).

Table 4. Canonical coefficients, intraset correlations, and coefficients of determination (r^2) between environmental variables and CCA axes 1–3.

	Canonical coefficients			Intraset correlations			r^2		
Environmental variable	1	2	3	1	2	3	1	2	3
Fathead minnows	–0.18	–0.09	–0.02	–0.97	–0.18	0.18	0.97	0.21	0.23
Aquatic plants	–0.02	–0.12	–0.14	0.55	–0.76	–0.35	0.58	0.70	0.39
Average depth	–0.06	0.05	–0.14	–0.40	0.78	–0.48	0.36	0.76	0.51

whereas wetlands with fathead minnows were characterized by higher abundance of Corixidae.

Discussion

The results of cluster analysis, PCA, and CCA were very similar, indicating that fathead minnows have key roles in structuring aquatic invertebrate communities in semipermanent prairie wetlands. This single variable had a stronger influence than any other that we considered. Presence of fathead minnows results in a distinctly different invertebrate community due to lower abundance of a number of invertebrate taxa, whereas only Corixidae responded positively to these fish.

Percent variance explained by the first three CCA axes appears low, but this is often the case with ecological data (ter Braak and Verdonschot 1995). The importance of axes should not be judged by percent variance explained but rather by size of the eigenvalues, the results of Monte Carlo tests, and the potential for ecological interpretation (ter Braak 1995; ter Braak and Verdonschot 1995). Eigenvalues for all three axes in this study were significant, and CCA identified abundance of fathead minnows, wetland depth, and abundance of aquatic plants as significant influences on invertebrate community structure.

Lowered abundance of most taxa likely results from predation by fathead minnows, as most of the taxa found to be significant indicator species in our analysis have been reported to be important in the diet of fathead minnows (Held and Peterka 1974; Price et al. 1991). Exceptions are Dytiscidae adults and larvae, Notonectidae, Anisoptera, Zygoptera, and Hirudinea. These taxa are all predators on aquatic invertebrates (Bay 1974) and may be less abundant in wetlands with minnows due to lower abundance of prey. It is also plausible that these taxa have not been observed previously in diet analyses because they are consumed much less frequently than other invertebrates.

Invertebrate communities appear to be extremely sensitive to the presence of fathead minnows. Abundance of fathead minnows varied considerably in our study wetlands with fish, with the highest seasonal average being 20 times that of the lowest. However, PCA indicated that invertebrate communities in wetlands with fathead minnows did not vary gradually along a gradient of increasing fathead minnow abundance. Instead, they occurred as a discrete group, distinctly separated from the invertebrate communities found in fishless wetlands. This indicates that the relationship between fathead minnow abundance and invertebrate community structure is nonlinear; even at relatively low densities, fathead minnows have a large influence on invertebrates.

Densities of fathead minnows in early spring appear to be highly variable (Duffy 1998), but populations often may be reduced by partial winterkill (Held and Peterka 1974). Our results indicate that these lowered populations of fathead minnows may still have an influence on invertebrate communities in early spring when waterfowl and other organisms are most dependent on this food resource. Additionally, consistent human introduction or immigration of even small numbers of fathead minnows into wetlands too shallow to sustain a population from year to year, such as seasonal wetlands, could have important impacts on invertebrate communities. Diversion of seasonally high water from deeper wetlands via ditches or installation of culverts may integrate formerly isolated seasonal wetlands with deeper semipermanent wetlands and lakes. Thus, seed populations of fathead minnows may be introduced into seasonal wetlands year after year, limiting development of invertebrate communities expected to characterize these sites.

Although fathead minnows were the most important variable that we identified, depth and abundance of aquatic plants were also related to invertebrate community structure.

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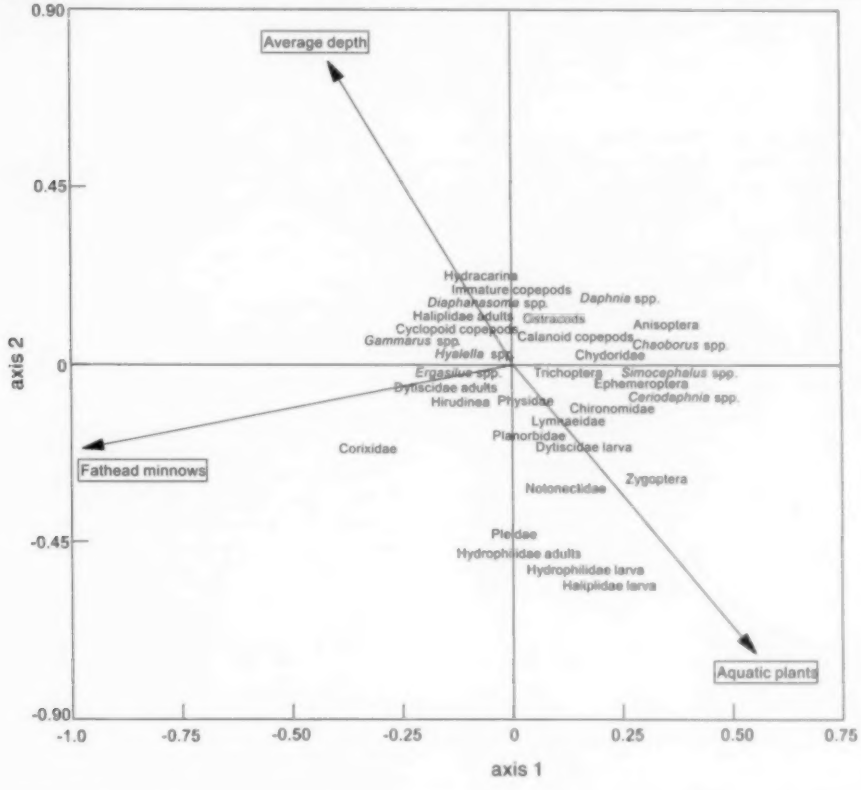
Fig. 4. Results of CCA (axes 1 and 2 species scores) for invertebrate taxa and environmental data in 19 wetlands sampled in 1996 and 1997. Vectors (arrows) point in the direction of increasing values for the respective variables, with longer vectors indicating stronger correlations between vectors and axes. Coefficients of determination for the three environmental variables are the same as in Fig. 3.



CCA ordination scores indicated that a number of invertebrates were positively associated with high abundance of aquatic plants and shallow average depth. Although the relationship varies from taxon to taxon, it appears that shallow wetlands with abundant plants favor higher abundance of many aquatic invertebrates that we considered. Invertebrates may benefit from decreased risk of predation (Crowder and Cooper 1982), increased habitat complexity (Crowder and Cooper 1982), and higher food resources (Carpenter and Lodge 1986) associated with stands of submerged plants.

Surface area, abundance of amphibians, and wetland history (restored versus natural) were not significantly related to the structure of invertebrate communities at our sites. Predation by larval salamanders influences the structure of invertebrate communities in microcosms (Holomuzki et al. 1994), and Carlson and Berry (1990) reported that densities of larval salamanders were similar to those of fathead minnows in four South Dakota wetlands. However, our results indicate that salamanders had less influence on invertebrate communities than did wetland depth and abundances of fathead minnows and aquatic plants.

Ordination also provides information on relationships among environmental variables. Our CCA data revealed inverse relationships between abundances of fathead minnows and aquatic plants along axis 1 and between depth and plant abundance along axis 2. Directions of these vectors indicate that wetlands with fathead minnows will have lower abundance of plants compared with fishless wetlands of similar depth. Abundance of submerged aquatic plants is heavily influenced by light availability, which in turn depends on both depth and turbidity (Scheffer et al. 1993). Presence of fish has been associated with increased turbidity in other studies (Spencer and King 1984; Hanson and Butler 1994; and many others), and we found turbidity to be higher in wetlands with fathead minnows compared with fishless sites (K.D. Zimmer, M.A. Hanson, and M.G. Butler, unpublished data). Thus, the negative relationship between fathead minnows and aquatic plants probably results from higher turbidity in wetlands with fathead minnows. However, this is undoubtedly mediated by wetland depth, as the inverse relationship between aquatic plants and depth was stronger than the relationship between fathead minnows and aquatic plants.

Ordination also showed that the abundance of fathead minnows was not strongly correlated with wetland depth; thus, the abundance of these fish in semipermanent wetlands can not be accurately predicted by depth. Survival of fathead minnows in shallow wetlands during winter is facilitated by behavioral and physiological characteristics, including movement to areas with higher oxygen concentrations

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(Peterka 1989) and possible use of anaerobic metabolic pathways (Klinger et al. 1982). Other factors, such as recolonization from neighboring waters during spring runoff, may also reestablish populations after complete winterkills.

Fathead minnows play predominate roles in structuring invertebrate communities in semipermanent prairie wetlands. Such influences, in turn, impact other wetland-dependent species and result in functionally different food webs and nutrient dynamics (Bouffard and Hanson 1997). From a wetland-management perspective, lower invertebrate abundance probably reduces the suitability of some wetlands as habitat for amphibians, waterfowl, and other animals that forage on invertebrates. Competition between these species and fathead minnows may be especially severe in low-rainfall years or in regions where semipermanent wetlands (the type most commonly supporting fathead minnow populations) are the only available habitat.

Fish predation on zooplankton communities usually reduces the numbers of large-bodied species due to size-selective predation (Carpenter and Kitchell 1993). However, fathead minnows influenced the abundance of several zooplankton taxa, including large- (*Daphnia* spp.) and small-bodied (chydorid) cladocerans, as well as both calanoid and cyclopoid copepods. The wide diversity of zooplankton affected is probably due to the foraging flexibility of fathead minnows, as they utilize both particulate- and filter-feeding strategies (Hambright and Hall 1992). Because they consume both large- and small-bodied prey, fathead minnows are likely to have different impacts on invertebrate communities than do fish species that rely on particulate feeding only (Hambright and Hall 1992). In addition to their broad impacts on zooplankton taxa, fathead minnows also affect the abundance of macroinvertebrates in wetlands. This finding is important because it indicates that populations of fathead minnows in wetlands exert broad influences on aquatic invertebrate communities, beyond the scope of pelagic trophic interactions that have been so well documented in lake zooplankton communities.

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References

Bay, E.C. 1974. Predator–prey relationships among aquatic insects. Annu. Rev. Entomol. **19**: 441–453.

Bellrose, F.C., and Low, J.B. 1978. Advances in waterfowl management research. Wildl. Soc. Bull. **6**: 63–72.

Biondini, M.E., Mielke, P.W., and Berry, K.J. 1988. Data-dependent permutation techniques for the analysis of ecological data. Vegetatio, **75**: 161–168.

Bouffard, S.H., and Hanson, M.A. 1997. Fish in waterfowl marshes: waterfowl managers' perspective. Wildl. Soc. Bull. **25**: 146–157.

Carlson, B.N., and Berry, C.R. 1990. Population size and economic value of aquatic bait species in palustrine wetlands of eastern South Dakota. Prairie Nat. **22**: 119–128.

Carpenter, S.R., and Kitchell, J.F. 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge, England.

Carpenter, S.R., and Lodge, D.M. 1986. Effects of submersed macrophytes on ecosystem processes. Aquat. Bot. **26**: 341–370.

Cox, B.B., Jr., Hanson, M.A., Roy, C.R., Euliss, N.H., Jr., Johnson, D.H., and Butler, M.G. 1998. Growth and survival of mallard ducklings in relation to aquatic invertebrates. J. Wildl. Manage. **62**: 124–133.

Crowder, L., and Cooper, W. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology, **63**: 1802–1813.

Depp, E.R., and Lathrop, R.C. 1992. A comparison of two rake sampling techniques for sampling aquatic macrophytes. Wis. Dep. Nat. Resour. Res. Manage. Find. No. 32.

Dieter, C.D., Duffy, W.G., and Flake, L.D. 1996. The effect of phorate on wetland macroinvertebrates. Environ. Toxicol. Chem. **15**: 308–312.

Duffy, W.G. 1998. Population dynamics, production, and prey consumption of fathead minnows (*Pimephales promelas*) in prairie wetlands: a bioenergetic approach. Can. J. Fish. Aquat. Sci. **55**: 15–27.

Dufrene, M., and Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. **67**: 345–366.

Euliss, N.H., Jr., Jarvis, R.L., and Gilmer, D.S. 1991. Standing crops and ecology of aquatic invertebrates in agricultural drainwater ponds in California. Wetlands, **11**: 179–190.

Euliss, N.H., Jr., Wrubleski, D.A., and Mushet, D.M. 1999. Wetlands of the prairie pothole region: invertebrate species composition, ecology, and management. *In* Invertebrates in freshwater wetlands of North America: ecology and management. *Edited by* D.P. Batzer, R.P. Rader, and S.A. Wissinger. John Wiley & Sons, New York.

Fisher, R.A. 1958. Statistical methods for research workers. 13th ed. Hafner, New York.

Hambright, K.D., and Hall, R.O. 1992. Differential zooplankton feeding behaviors, selectivities, and community impacts of two planktivorous fishes. Environ. Biol. Fishes, **35**: 401–411.

Hanson, M.A., and Butler, M.G. 1994. Responses of plankton, turbidity, and macrophytes to biomanipulation in a shallow prairie lake. Can. J. Fish. Aquat. Sci. **51**: 1180–1188.

Hanson, M.A., and Riggs, M.R. 1995. Effects of fish predation on wetland invertebrates: a comparison of wetlands with and without fathead minnows. Wetlands, **15**: 167–175.

Held, J.W., and Peterka, J.J. 1974. Age, growth, and food habits of the fathead minnow, *Pimephales promelas*, in North Dakota saline lakes. Trans. Am. Fish. Soc. **103**: 743–757.

Holomuzki, J.R., Collins, J.P., and Brunkow, P.E. 1994. Trophic control of fishless ponds by tiger salamander larvae. Oikos, **71**: 55–64.

Klinger, S.A., Magnuson, J.J., and Gallepp, G.W. 1982. Survival mechanisms of the central mudminnnow (*Umbra limi*), fathead minnow (*Pimephales promelas*), and brook stickleback (*Culaea*

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inconstans) for low oxygen in winter. Environ. Biol. Fishes, **7**: 113–120.

Litvak, M.K., and Mandrak, J.F. 1993. Ecology of freshwater bait fish in Canada and the United States. Fisheries (Bethesda), **18**: 6–13.

Ludwig, J.A., and Reynolds, J.F. 1988. Statistical ecology: a primer on methods and computing. John Wiley & Sons, New York.

McCune, B. 1997. Influence of noisy environmental data on canonical correspondence analysis. Ecology, **78**: 2617–2623.

McCune, B., and Mefford, M.J. 1997. Multivariate analysis of ecological data. Version 3.0. MjM Software, Gleneden Beach, Oreg.

Murkin, H.R., and Wrubleski, D.A. 1988. Aquatic invertebrates of freshwater wetlands: function and ecology. *In* The ecology and management of wetlands. Vol. 1. Ecology of wetlands. *Edited by* D.D. Hook et al. Croom Helm, London, England.

Murkin, H.R., Abbott, P.G., and Kadlec, J.A. 1983. A comparison of activity traps and sweep nets for sampling nektonic invertebrates in wetlands. Freshwater Invertebr. Biol. **2**: 99–106.

Neckles, H.A., Murkin, H.R., and Cooper, J.A. 1990. Influences of seasonal flooding on macroinvertebrate abundance in wetland habitats. Freshwater Biol. **23**: 311–322.

Palmer, M.W. 1993. Putting things in even better order: the advantages of canonical correspondence analysis. Ecology, **74**: 2215–2230.

Payer, R.D., and Scalet, C.G. 1978. Population and production estimates of fathead minnows in a South Dakota prairie wetland. Prog. Fish-Cult. **40**: 63–66.

Peterka, J.J. 1989. Fishes in northern prairie wetlands. *In* Northern prairie wetlands. *Edited by* A. van der Valk. Iowa State University Press, Ames, Iowa. pp. 302–315.

Pettigrew, C.T., Hann, B.J., and Goldsborough, L.G. 1998. Waterfowl feces as a source of nutrients to a prairie wetland: responses of microinvertebrates to experimental additions. Hydrobiologia, **362**: 55–66.

Power, M.E. 1990. Effects of fish in river food webs. Science (Washington, D.C.), **250**: 811–814.

Price, C.J., Tonn, W.M., and Paszkowski, C.A. 1991. Intraspecific patterns of resource use by fathead minnows in a small boreal lake. Can. J. Zool. **69**: 2109–2115.

Rice, W.R. 1990. A consensus combined *P*-value test and the family-wide significance of component tests. Biometrics, **46**: 303–308.

Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B., and Jeppesen, E. 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. **8**: 275–279.

Spencer, C.N., and King, D.L. 1984. Role of fish in regulation of plant and animal communities in eutrophic ponds. Can. J. Fish. Aquat. Sci. **41**: 1851–1855.

Stewart, R.E., and Kantrud, H.A. 1971. Classification of natural ponds and lakes in the glaciated prairie region. U.S. Fish Wildl. Serv. Res. Publ. No. 92.

Swanson, G.A. 1978. A plankton sampling device for shallow wetlands. J. Wildl. Manage. **42**: 670–672.

Szalay, F.A., and Resh, V.H. 1997. Responses of wetland invertebrates and plants important in waterfowl diets to burning and mowing of emergent vegetation. Wetlands, **17**: 149–156.

ter Braak, C.J.F. 1995. Ordination. *In* Data analysis in community and landscape ecology. *Edited by* R.H.G. Jongman, C.J.F. ter Braak, and O.F.R. van Tongeren. Cambridge University Press, Cambridge, England. pp. 91–173.

ter Braak, C.J.F., and Verdonschot, P.F.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. **57**: 255–289.

van Tongeren, O.F.R. 1995. Cluster analysis. *In* Data analysis in community and landscape ecology. *Edited by* R.H.G. Jongman, C.J.F. ter Braak, and O.F.R. van Tongeren. Cambridge University Press, Cambridge, England. pp. 174–212.

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An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques

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Abstract: We tested the hypothesis that phytoplankton productivity in pelagic Lake Erie is limited by low Fe bioavailability during the period of thermal stratification. Iron enrichment (20 and 200 nM Fe) of water sampled from the eastern basin surface water (5 m depth) in July 1996 revealed a dramatic 180 and 30% increase in the standing crop of the picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) size fractions, respectively. Light-saturated rates of photosynthesis for picoplankton increased 2.8 times that of the controls within 24 h. In contrast, simultaneous P and Fe enrichment experiments carried out in July 1997 and August 1998 with water samples from three pelagic stations revealed that P enrichment alone stimulated phytoplankton growth, although at several stations, phytoplankton yield was greater in combined P- and Fe-amended experiments relative to P-enriched treatments. Periodogram analysis of meteorological buoy wave height data and heat budgets calculated from thermal profiles in the eastern basin revealed important interyear differences in precruise conditioning of the epilimnion. Low-frequency, turbulent mixing events that characterized the 30-day precruise period in the years 1997 and 1998 relative to 1996 may have prevented Fe-stressed conditions in the phytoplankton community studied in 1997 and 1998.

Résumé : L'hypothèse que la production phytoplanctonique est limitée par la faible disponibilité du Fe lors de la stratification thermique au lac Érié a été étudiée. Suivant un enrichissement (20 et 200 nM Fe) d'échantillons de surface (5 m) provenant du bassin Est effectué en juillet 1996, nous avons observé une augmentation de 180 et 30% de la biomasse picoplanctonique (0,2–2 μm) et nanoplanctonique (2–20 μm) respectivement. La photosynthèse maximale picoplanctonique a augmenté d'un facteur 2.8 par rapport à l'échantillon témoin durant les premières 24 h. Des expériences d'enrichissement simultané en P et en Fe effectuées en juillet 1997 et en août 1998 à trois stations pélagiques ont révélé que seul le P stimulait la croissance, bien qu'à quelques stations, la biomasse phytoplanctonique était plus élevée dans les traitements enrichis avec le P et le Fe. L'analyse par périodogramme de la hauteur des vagues obtenue d'une bouée météorologique ainsi que le bilan de chaleur déterminé à partir des profiles thermiques ont révélé d'importantes différences inter-annuelles sur une période de 30 jours précédent les croisières. Il est possible que des événements de fort mélange turbulent en 1997 et 1998 par rapport à 1996 aient empêché une carence en Fe chez les populations phytoplanctoniques étudiées en 1997 et 1998.

Introduction

Only recently have trace metal clean techniques been used to reliably and accurately measure the concentration of trace metals in surface waters of the Great Lakes (Coale and Flegal 1989; Nriagu et al. 1993, 1996). Whereas some metals reveal distributions suggesting anthropogenic sources, i.e., increasing in concentration from the upper to lower lakes, e.g., Tl (Cheam et al. 1995) and Cr (Beaubien et al. 1994), the dissolved concentrations of some physiologically essential, bioactive trace metals approach values measured in open oceanic surface waters. For example, Zn concentrations in pelagic Lake Erie surface waters during thermal stratification have been measured in the range of 0.39–0.84 nM (Coale and Flegal 1989); a representative surface water Zn concentration in the North Pacific Ocean is 0.23 nM (Bruland et al. 1994). The concentrations of bioactive trace metals are so low in the surface waters of some regions of the Great Lakes that limitation of phytoplankton growth due to the low bioavailability of trace metals is hypothesized. Such is the case for Fe.

The discovery that Fe additions enhance phytoplankton growth in the equatorial Pacific Ocean (Martin et al. 1994; Coale et al. 1996) and in seawater from subtropical regions of the world's oceans (Martin and Fitzwater 1988) has greatly stimulated oceanographic interest in the bioavailability of this element. Given the low solubility (K_{sp} =

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Table 1. Chlorophyll *a*, total dissolved Fe (<0.2 μm), and calculated free Fe^{3+} concentrations in Lake Erie surface waters.

Date	Station	Latitude	Longitude	Chl *a* (μg·L^{-1})	Fe (nM)	pFe (–log Fe^{3+} (mol·L^{-1}))
July 1996	935	42°35′33″	79°27′58″	0.90	19.9	20.9
July 1996	23	42°30′26″	79°53′54″	0.97	16.2	21.0
July 1996	946	42°09′55″	80°38′30″	1.14	7.3	21.4
July 1996	953	42°11′09″	81°26′26″	—	21.1	20.9
July 1996	84	41°55′57″	81°39′35″	0.67	8.1	21.3
July 1996	341	41°47′11″	82°17′10″	0.61	3.0	21.8
July 1996	357	41°49′32″	80°58′26″	2.23	17.7	21.0
July 1997	935	42°35′33″	79°27′58″	1.47	33.8	20.7
July 1997	23	42°30′26″	79°53′54″	2.76	11.8	21.1
July 1997	953	42°11′09″	81°26′26″	1.41	5.1	21.5
August 1998	935	42°35′33″	79°27′58″	2.20	11.9	21.1
August 1998	23	42°30′26″	79°53′54″	2.26	8.3	21.3
August 1998	84	41°55′57″	81°39′35″	1.79	4.5	21.6

Note: pFe was calculated using a computerized chemical speciation model (see text for details).

Table 2. Summary of experimental conditions in Lake Erie, 1996–1998.

Year	Lake Erie station(s)	Prescreening size (μm)	Experimental treatment	Sample incubation
1996	23	20	Control +20 nM Fe +200 nM Fe	Incubator
1997	935, 23, 953	20	Control +20 nM Fe +200 nM Fe +200 nM P +200 nM P + 20 nM Fe +200 nM P + 200 nM Fe	Incubator
1998	935, 23, 84	500	Control +50 nM Fe +200 nM P +200 nM P + 50 nM Fe +1 μM EDTA +1 μM EDTA + 50 nM Fe	Deck box

10^{-20}) of Fe^{3+}, the complex aqueous redox chemistry, and the high degree of organic complexation of Fe (99.97% organically bound; Rue and Bruland 1995), there may be kinetic constraints on Fe bioavailability that limit phytoplankton growth (Rich and Morel 1990). It is therefore not surprising that Fe limitation has been found in pelagic ocean environments where the concentration of total dissolved Fe (0.08–0.2 nM; Martin and Gordon 1988; Rue and Bruland 1995) is a great deal lower than in pelagic Lake Erie (3–34 nM) (Table 1). However, it is the bioavailability of Fe to phytoplankton in Lake Erie that is important. Based on chemical speciation calculations (see Results), we hypothesized that Fe bioavailability in Lake Erie is low enough to induce physiological limitation of phytoplankton growth during the period of thermal stratification. To test this hypothesis, in 1996 and 1997, trace metal clean protocols were used to conduct Fe enrichment experiments designed to assess the response of autotrophic members of the picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) sampled from the eastern and central basins of Lake Erie. In 1998, similar experiments were conducted and included microplankton (20–500 μm).

Materials and methods

Trace metal clean protocols

Precautions were taken to avoid contamination of sampled lake water during sampling and the manipulation steps that followed. The cleaning protocol for polycarbonate and Teflon® plasticware involved a warm soap wash (Liqui-Nox, 1%), methanol (HPLC grade) soak, and HCl (Analar® trace metal grade, 10%) soak for 24–48 h with a sevenfold rinse after each cleaning step using deionized water (>17.5 MΩ·cm^{-1}). An 8-L Teflon®-coated sampling bottle (Go-Flo, model 1080, General Oceanics, Miami, Fla.) was used to collect water. The sampling bottle was treated similarly except that 1% HCl was used in the acid leaching step (the use of 1% acid was used as a precaution to prevent damage to the Viton and silicone O-rings). All polycarbonate filters and nylon screens were previously soaked in 10% HCl and exhaustively rinsed with deionized water before use. Water samples collected for trace metal analysis were stored in Teflon® bottles.

Enrichment experiments

Over the 3-year study period, the experimental design was modified as described below and is summarized in Table 2. During 1996 and 1997, we designed the enrichment experiments to specifically examine the growth of nanoplankton and picoplankton. Given the abundance of *Microcystis* captured on a 20-μm-mesh nylon screen in 1997, we decided to include this size fraction in the 1998 study, so prescreening was carried out on a 500-μm-mesh nylon screen to remove only large crustacean herbivores. The enrichment scheme initially involved low and high Fe additions (1996) and later included P-amended (1997) and EDTA- and Fe+EDTA-amended treatments (1998, see below).

Lake water was collected from multiple sample bottle casts on a Kevlar® line to a depth of 5 m (1996, 1997) or 10 m (1998) at each station. All further manipulations were conducted in a portable clean room onboard the research vessel (the clean room was

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equipped with an airlock and a HEPA-filtered inner sanctum containing a Class-100 laminar flow hood. All manipulations, including subsampling of lake water and experimental treatment bottles, took place in the clean room environment). Lake water was screened through an acid-cleaned screen-type filter, pooled in a polycarbonate carboy, and dispensed under pressure from prefiltered (<0.2 μm), prepurified N_2 into 2-L polycarbonate bottles. In 1996 and 1998, Fe stock solutions (0.72 mM $FeCl_2$-$4H_2O$ in deionized water, pH 2) were prepared 48 h prior to cruise departure from the same crystalline stock that was initially verified for P contamination (1.4×10^{-5} mol P·mol Fe^{-1}) and used over the 3-year period. Stock solutions were verified after the cruises by AAS (total Fe) and were within 2% of nominal values. Verifications with ferrozine, a Fe(II)-specific complexant, revealed that Fe remained reduced for at least 1 month in these stock solutions. In 1997, a small amount of the crystalline stock was preweighed in the laboratory and rehydrated aboard the ship; visual inspection of the crystals prior to rehydration revealed that some surface oxidation of the Fe(II) had occurred, although postcruise total Fe measurements by AAS were within the nominal value. Enrichment experiments may have contained a small amount of oxidized Fe in 1997.

In 1996, Fe was added to bottles to give low (20 nM) and high (200 nM) treatments. In 1997, additional experimental treatments included a phosphate-enriched (200 nM) treatment and phosphate-enriched low-Fe (200 nM P + 20 nM Fe) and high-Fe (200 nM P + 200 nM Fe) treatments. Phosphate (K_2HPO_4) was added from a stock solution that was previously passed through an ion-exchange column (Chelex-100, Na-form, Biorad) and irradiated by ultraviolet light (12 h, 1000 W) to eliminate trace metal and organic contaminants, respectively. In 1998, 1 μM EDTA was added alone or in conjunction with Fe to determine if chelated (i.e., solubilized) Fe might stimulate growth and to examine possible limitation and (or) toxicity alleviation by other trace metals. All treatments were conducted in duplicate and two bottles received no additions and thus served as controls. Bottles were incubated in environmental chambers in 1996 and 1997 under simulated in situ conditions: 20°C and light provided by fluorescent tubes (190 μmol photons·m^{-2}·s^{-1} on a 12 h light : 12 h dark cycle. In 1998, incubations were carried out in translucent closed polypropylene deck boxes continuously cooled with surface lake water; a layer of neutral-density netting reduced the incident light by 52%. At approximately 12- to 24-h intervals, subsamples were removed from the bottles for measurements of size-fractionated chlorophyll *a* (Chl *a*) concentrations and light-saturated rates of photosynthesis.

Assessing treatment response

Chlorophyll a content

Changes in Chl *a* concentration were chosen as surrogates for changes in phytoplankton biomass and used to monitor growth in the experimental bottles over time. For Chl *a* determinations, 100 mL was removed from each bottle and serially filtered onto a 2-μm polycarbonate membrane filter (47 mm in diameter, Nuclepore) followed by a 0.2- or 0.4-μm filter (1996 only). Chlorophyll *a* retention by 0.2- and 0.4-μm filter pore sizes was identical. Filters were immediately plunged into cold 90% acetone and allowed to extract overnight at 4°C. After centrifugation, pigment concentration was determined by fluorometric analysis (Welschmeyer 1994) aboard the research vessel.

Photosynthesis

Light-saturated rates of photosynthesis were measured to examine changes in physiological status in the phytoplankton community. If phytoplankton growth is limited by the availability of Fe, then photosynthesis will also be limited by reason of reduced Chl *a* production and a reduction in the levels of cytochromes and complementary proteins such as ferredoxin that are intimately involved in photosynthesis (Raven 1988).

To determine light-saturated photosynthetic rates, 125-mL subsamples from each bottle were placed into acid-cleaned 125-mL borosilicate glass-stoppered bottles. $NaH^{14}CO_3$ was added into each bottle to give a total ^{14}C radioactivity of 141 kBq. These bottles were incubated adjacent to the enriched 2-L bottles for 2 h where they experienced the same irradiance field, which was close to midday in the deck boxes. After the incubation period, the entire bottle contents were serially filtered onto a 2-μm filter and then a 0.2-μm filter. Filters were rinsed with 10 mL of filter-sterilized lake water. Time-zero filter and dark bottle controls revealed that there was negligible nonphotosynthetic sorption of ^{14}C by particles. All filter samples containing ^{14}C were immediately frozen for transport to the laboratory. Filters were counted by liquid scintillation spectrophotometry (Wallac Winspectral, model 1414) and quench corrected using an internal quench library.

Iron accumulation by plankton

Radioactive Fe was used to trace the uptake of this element from the aqueous to particulate phase in the sampled lake water. The accumulation of Fe by plankton was conducted on water sampled from Stations 935, 23, and 953 in 1997. Details of this protocol are provided elsewhere (Twiss and Campbell 1998). Briefly, radiolabelled Fe (12 μL of $^{59}FeCl_2$ in 0.1 M HCl) was added to triplicate bottles, each containing 2 L of sampled lake water (filtered <20 μm, see above) to give a total radioactivity of 222 kBq and a nominal added Fe concentration of 2.2 nM. Bottles were incubated under simulated in situ conditions (see above), and at timed intervals over a period of 24–60 h, the partitioning of ^{59}Fe into the picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) was determined by filtration. Two 50-mL aliquots from each bottle were placed in a polyethylene tube to which Na_2EDTA (pH 8) was added to give a concentration of 10 μM EDTA. After a period of 20 min, the contents of each tube were filtered onto a 0.2- or 2-μm pore size filter (47 mm in diameter, Nuclepore). Radioactivity was measured using liquid scintillation spectrophotometry (Wallac Winspectral, model 1414). Accumulated ^{59}Fe in the picoplankton-sized fraction was determined by difference, i.e., $[^{59}Fe_{0.2-2\,\mu m}] = [^{59}Fe_{>0.2\,\mu m}] - [^{59}Fe_{>2\,\mu m}]$, and accumulation in each size fraction was expressed as the percentage of the total aqueous ^{59}Fe measured in duplicate 1-mL aliquots removed from each bottle at each sampling period.

We consider the use of the 20-min EDTA wash to be effective at removing adsorbed Fe from cell and detrital surfaces under these conditions ([Ca] = $10^{-3.1}$, pH 8). The Ti(III)–citrate–EDTA wash technique (Hudson and Morel 1989) has been used in marine studies as an indicator of intracellular Fe, i.e., any Fe remaining in particulate form is inert to reductive dissolution. The titanium wash technique was developed because of slow dissociation kinetics of the Ca–EDTA complex, which dominates EDTA speciation in seawater ([Ca] = $10^{-2.0}$, pH 8) and effectively lowers the reaction kinetics between EDTA and other trace metals (Morel and Hering 1993). An earlier phycological study revealed no difference when using 1 mM EDTA or stronger reductants (10 mM ascorbic acid or 1 mM EDTA – ascorbic acid) in complexing extracellular Mn and Fe (Schenck et al. 1988) in freshwater.

Trace metal analyses

Total dissolved Fe analysis

In 1996, lake water was filtered (<0.2 μm) and acidified to pH <2 using HCl (Merck Supra-Pur; final concentration 0.04 M). Iron was analyzed by graphite furnace atomic absorption spectrophotometry (Varian SpectraAA model 300) using an $Mg(NO_3)_2$ matrix modifier and standard additions methodologies. In 1997,

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lake water was filtered (<0.2 µm) and stored frozen; prior to analysis, water was acidified to pH <2 using HNO_3 (Ultrex II, J.T. Baker, final concentration 0.05 M). Analysis of Fe content was conducted by inductively coupled plasma mass spectrometry (Finnegan Matt Element) using a standard curve and indium as an internal standard. In 1998, lake water was filtered (<0.2 µm) and acidified (HNO_3 Ultrex II, final concentration 0.005 M). Iron was analyzed by graphite furnace (Perkin-Elmer SIMAA-6000) using multiple injection analysis and $Mg(NO_3)_2$ as a matrix modifier.

Iron speciation

Aqueous Fe speciation was determined using a computerized chemical speciation model (Windermere Humic Aqueous Model–Waters (WHAM), version 1.0; Tipping 1994). Calculations were based on reported major ion concentrations in the epilimnion of Lake Erie during summer (Rockwell et al. 1989), dissolved concentrations of Fe determined in this study (see Table 1), a dissolved humic substance concentration of 1.6 $mg \cdot L^{-1}$ (9:1 ratio of fulvic acid to humic acid), a pH of 8, and a temperature of 20°C. The concentration of dissolved organic C (DOC) is relatively constant throughout Lake Erie surface waters (3.2 ± 0.5 $mg \cdot L^{-1}$, mean ± SD) during the summer months (July 1994 and August 1995) (S. L'Italien, Environment Canada, National Water Research Institute, Burlington, Ont., personal communication). For the purpose of the calculation, we have assumed that humic and fulvic acids comprise 50% of the DOC (Buffle 1988) present in the surface waters of the lake.

Statistical analyses

One-way repeated measures ANOVA was performed and an a posteriori comparison of means (biomass content or photosynthesis activity) of treatments was conducted with the Tukey test (SigmaStat, SPSS Corp.) after verifying the normality and heteroscedasticity of the data.

Results

Dissolved Fe analyses

Total dissolved Fe values were variable (Table 1). The range in Fe concentrations measured in the surface waters of the eastern and central basins of Lake Erie during the 1996 and 1997 survey (3–34 nM) (Table 1) are close to the range of measurements reported for these basins by other researchers using trace metal clean sampling protocols (2–15 nM; Nriagu et al. 1996).

Dissolved Fe represented a small fraction of total aqueous Fe. In 1996, total Fe was determined in samples by acidifying unfiltered lake water at two stations. Total Fe was 30.6 nM (79% particulate Fe) at Station 84 (central basin) and 485 nM (96% particulate Fe) at Station 357 (western basin). A temperature–depth profile at Station 357 revealed that the surface water of the western basin was in contact with the sediment; hence the elevated Fe concentration is probably due to suspended sediments. All other stations analyzed in the current study were epilimnetic waters.

Measured dissolved Fe concentrations were used to calculate the free ferric ion concentration at several stations in Lake Erie using the WHAM chemical speciation model (Tipping 1994). The model output revealed that most of the dissolved Fe is complexed with dissolved organic matter (>99%) (cf. Table 1) and the calculated free-ion concentrations are within the range known to cause Fe limitation in cyanobacteria (Wilhelm 1995) and eukaryotic microalgae (Brand 1991).

Iron enrichment assays

1996 results

In 1996, there was a profound stimulation of phytoplankton growth following the experimental addition of inorganic Fe to sampled surface water. Phytoplankton biomass in the picoplankton (0.4–2 µm) and nanoplankton (2–20 µm) size fractions increased following the addition of the Fe, relative to the control treatment (Figs. 1*a* and 1*b*). In the 20 nM Fe treatment, biomass increased by 182 and 30% in the picoplankton and nanoplankton size fractions, respectively.

The photosynthetic efficiency of the picoplankton in the Fe treatments increased dramatically within the first 24 h and returned to control levels within 3 days (Fig. 1*c*), whereas there was little change in the photosynthetic efficiency of the nanoplankton in the same treatments (Fig. 1*d*) over the 3-day incubation. The results suggest that the increased photosynthetic capacity of the picoplankton caused by the addition of Fe was rapidly translated into picoplankton biomass.

1997 and 1998 results

The addition of Fe alone in 1997 and 1998 did not result in any significant growth of nanoplankton or picoplankton at any of the studied stations (Tables 3 and 4). In 1997 at nearshore Station 935, phytoplankton biomass increased significantly in all P-enriched treatments relative to unenriched samples in both phytoplankton size fractions (Table 3). There was no increased growth in the presence of added Fe, suggesting that P was limiting to all size fractions at this station. At Stations 23 and 953, the combined addition of Fe and P was required to significantly stimulate growth in the nanoplankton size fraction, whereas P amendments alone resulted in increased growth of the picoplankton size fraction at Station 23 (results were close to being significant at Station 953). These observations suggest that both P and Fe were colimiting to nanoplankton at these stations, whereas picoplankton appear to be mostly P limited.

A marked increase in photosynthetic efficiency in the picoplankton in the Fe-enriched treatments was not observed in 1997 as it was in 1996. By the 48-h time point, the assimilation ratios of both picoplankton and nanoplankton in the phosphate and the phosphate and Fe enrichment treatments had doubled relative to the control and Fe-enriched values at Stations 23 and 953 (Table 5; measurements were not conducted at Station 935). Therefore, P addition alone appeared to stimulate light-saturated rates of photosynthesis in the 1997 assays.

In 1998, prefiltration on the coarser 500-µm-mesh size uncoupled changes in the the Chl *a* content from changes observed in the short-term ^{14}C assays. Major differences between the two measurements were observed, particularly for the microplankton size fraction, where biomass increase was not significant in any of the treatments at Stations 23 and 84 (Table 4), yet the short-term photosynthetic measurements revealed significant stimulation in all the P-enriched treatments relative to the control (Table 6). This suggests that this size fraction responded to P additions but that intense grazing activity by organisms <500 µm limited increases in biomass of the microzooplankton fraction (>20 µm).

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Fig. 1. Changes in (*a* and *b*) biomass and (*c* and *d*) photosynthetic efficiency of pelagic picoplankton and nanoplankton sampled at Station 23 in the eastern basin of Lake Erie following the addition of Fe in July 1996. Values are mean ± range/2, $n = 2$. ○, control; □, +20 nM Fe; △, +200 nM Fe.

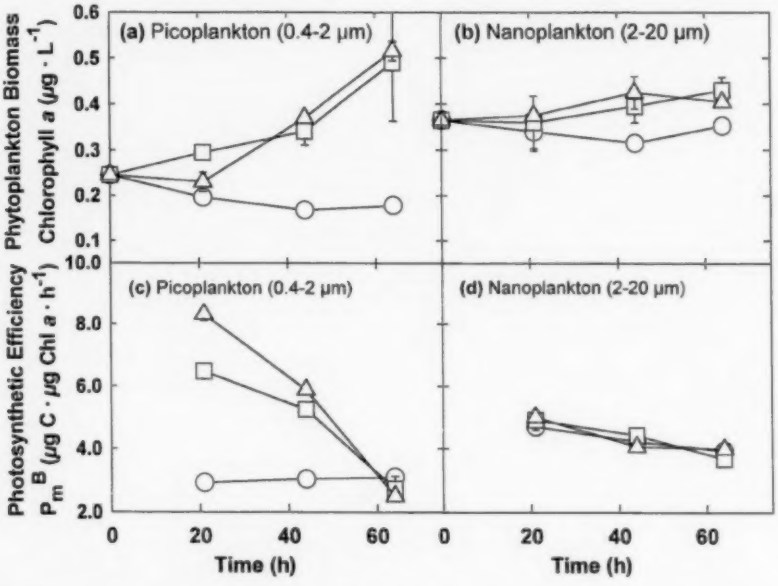
(a) Picoplankton (0.4-2 µm)
(b) Nanoplankton (2-20 µm)
(c) Picoplankton (0.4-2 µm)
(d) Nanoplankton (2-20 µm)
Phytoplankton Biomass Chlorophyll a (µg · L-1)
Photosynthetic Efficiency PmB (µg C · µg Chl a · h-1)
Time (h)
Time (h)

Table 3. Phytoplankton biomass measured in Fe and P enrichment experiments from surface water sampled from pelagic eastern (Stations 935 and 23) and central (Station 953) basins of Lake Erie during July 29 – August 1, 1997.

Treatment	Nanoplankton (2–20 µm) (µg Chl $a \cdot L^{-1}$)	Picoplankton (0.2–2 µm) (µg Chl $a \cdot L^{-1}$)
Station 935 (57 h)		
Control	0.84±0.01	0.45±0.05
20 nM Fe	0.89±0.04	0.49±0.06
200 nM Fe	0.90±0.15	0.43±0.00
200 nM PO_4-P	1.30±0.10*	0.83±0.01*
20 nM Fe + 200 nM PO_4-P	1.38±0.18*	0.98±0.07*
20 nM Fe + 200 nM PO_4-P	1.53±0.13*	1.15±0.05*
Station 23 (48 h)		
Control	1.53±0.03	0.37±0.01
20 nM Fe	1.65±0.05	0.44±0.01
200 nM Fe	1.52±0.06	0.37±0.01
200 nM PO_4-P	2.03±0.02	0.67±0.05*
20 nM Fe + 200 nM PO_4-P	2.45±0.15*	0.77±0.02*
200 nM Fe + 200 nM PO_4-P	2.55±0.25*	0.73±0.09*
Station 953 (45h)		
Control	1.49±0.01	0.59±0.01
20 nM Fe	1.50±0.08	0.67±0.03
200 nM Fe	1.45±0.03	0.58±0.02
200 nM PO_4-P	1.90±0.04	0.74±0.14
20 nM Fe + 200 nM PO_4-P	2.15±0.05*	0.76±0.04*
200 nM Fe + 200 nM PO_4-P	2.13±0.02*	0.68±0.00

Note: Values are treatment means (±range/2, $n = 2$) at the end of the stated incubation period. An asterisk indicates that the mean is significantly different from the control ($P = 0.05$).

In contrast, all P-enriched treatments had significantly more Chl *a* in the nanoplankton and picoplankton size fractions at all stations (Table 4) in 1998 except Station 84, where only the picoplankton growth was significantly stimulated by added P. However, visual inspection of netplankton hauls collected for another experiment revealed a much larger abundance of macrozooplankton at Station 84 compared with Stations 23 and 935, so increased grazing may

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Table 4. Phytoplankton biomass yield in Fe, P, EDTA, and Fe+EDTA enrichment experiments on surface water sampled from pelagic eastern (Stations 935 and 23) and central (Station 84) basins of Lake Erie during August 11–14, 1998.

Treatment	Microplankton (20–500 μm) (μg Chl $a \cdot L^{-1}$)	Nanoplankton (2–20 μm) (μg Chl $a \cdot L^{-1}$)	Picoplankton (0.2–2 μm) (μg Chl $a \cdot L^{-1}$)
Station 935 (71 h)			
Control	0.39±0.03	0.63±0.01	0.58±0.04
50 nM Fe	0.36±0.00	0.62±0.03	0.59±0.02
200 nM PO_4-P	0.60±0.03	1.27±0.17*	1.06±0.10*
50 nM Fe + 200 nM PO_4-P	0.54±0.03	1.17±0.01*	1.03±0.11*
1 μM EDTA	0.37±0.06	0.59±0.01	0.57±0.00
1 μM EDTA + 50 nM Fe	0.35±0.03	0.62±0.03	0.68±0.00
Station 23 (33 h)			
Control	0.35±0.05	0.53±0.02	0.68±0.04
50 nM Fe	0.28±0.01	0.54±0.04	0.67±0.04
200 nM PO_4-P	0.48±0.08	1.06±0.03*	1.15±0.15*
50 nM Fe + 200 nM PO_4-P	0.50±0.11	1.18±0.18*	1.23±0.18*
1 μM EDTA	0.32±0.06	0.53±0.01	0.75±0.00
1 μM EDTA + 50 nM Fe	0.29±0.02	0.62±0.01	0.78±0.00
Station 84 (42h)			
Control	0.28±0.00	0.40±0.02	0.32±0.00
50 nM Fe	0.33±0.05	0.42±0.02	0.40±0.00
200 nM PO_4-P	0.41±0.04	0.55±0.03	0.63±0.06*
50 nM Fe + 200 nM PO_4-P	0.42±0.06	0.67±0.11	0.67±0.09*
1 μM EDTA	0.26±0.00	0.42±0.01	0.39±0.01
1 μM EDTA + 50 nM Fe	0.28±0.01	0.43±0.03	0.40±0.01

Note: Values are treatment means (±range/2, $n = 2$) at the end of the stated incubation period. An asterisk indicates that the mean is significantly different from the control ($P = 0.05$).

Table 5. Photosynthetic assimilation ratios in phytoplankton biomass in Fe and P enrichment experiments on surface water sampled from pelagic eastern (Station 23) and central (Station 953) basins of Lake Erie during July 29 – August 1, 1997.

Treatment	Nanoplankton (2–20 μm) (μg Chl $a \cdot L^{-1}$)	Picoplankton (0.2–2 μm) (μg Chl $a \cdot L^{-1}$)
Station 23 (48 h)		
Control	2.52±0.09	1.78±0.02
20 nM Fe	2.72±0.06	1.96±0.17
200 nM Fe	2.85±0.28	1.81±0.15
200 nM PO_4-P	5.52±0.32*	4.02±0.12*
20 nM Fe + 200 nM PO_4-P	5.73±0.17*	4.13±0.25*
200 nM Fe + 200 nM PO_4-P	4.86±0.24*	3.77±0.31*
Station 953 (45h)		
Control	2.18±0.01	2.19±0.09
20 nM Fe	2.12±0.01	2.22±0.05
200 nM Fe	2.31±0.05	2.39±0.19
200 nM PO_4-P	2.79±0.33	2.95±0.35
20 nM Fe + 200 nM PO_4-P	2.82±0.13	3.25±0.26
200 nM Fe + 200 nM PO_4-P	2.98±0.14	3.29±0.03

Note: Values are treatment means (±range, $n = 2$) at the end of the stated incubation period. An asterisk indicates that the mean is significantly different from the control ($P = 0.05$).

have nullified increases in biomass in the nanoplankton size fraction, as discussed previously for the microplankton size fraction. This interpretation is supported by the increased light-saturated rates of photosynthesis (P_m^B) observed in all P-enriched samples and in all size fractions at Stations 23 and 84 in 1998 (Table 6). After a short 15-h incubation at Station 935, no differences among the treatments in any size fraction were observed.

Treatments enriched with EDTA and Fe+EDTA were indistinguishable from controls at all stations. This indicates that chelated Fe was not in short supply and suggests that other bioactive metals (e.g., Cu) were not present at toxic concentrations at the time of our experiments.

Iron accumulation

Despite the lack of growth stimulation by the addition of only Fe in the 1997 experimental treatments, Fe was in biological demand in the picoplankton and nanoplankton size

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Table 6. Photosynthetic assimilation ratios measured in Fe, P, EDTA, and Fe+EDTA enrichment experiments from surface water sampled from pelagic eastern (Stations 935 and 23) and central (Station 84) basins of Lake Erie during August 10–14, 1998.

Treatment	P_m^B microplankton (20–500 μm) (μg C·μg Chl a^{-1}·h^{-1})	P_m^B nanoplankton (2–20 μm) (μg C·μg Chl a^{-1}·h^{-1})	P_m^B picoplankton (0.2–2 μm) (μg C·μg Chl a^{-1}·h^{-1})
Station 935 (15 h)			
Control	1.04±0.04	4.44±0.05	6.05±0.04
50 nM Fe	0.78±0.03	4.07±0.05	5.55±0.59
200 nM PO_4-P	1.15±0.10	5.64±0.23	6.75±0.01
50 nM Fe + 200 nM PO_4-P	1.39±0.01	7.05±0.16	7.42±0.19
1 μM EDTA	1.06±0.08	4.73±0.27	5.56±0.34
1 μM EDTA + 50 nM Fe	0.87±0.05	4.03±0.59	5.63±0.36
Station 23 (33 h)			
Control	4.63±0.14	7.47±0.15	8.53±0.33
50 nM Fe	4.31±0.53	7.60±0.49	8.24±0.25
200 nM PO_4-P	6.96±1.06*	12.54±0.39*	11.60±0.72*
50 nM Fe + 200 nM PO_4-P	8.26±1.79*	12.65±1.63*	10.79±0.57*
1 μM EDTA	5.35±0.59	9.06±0.29	9.17±0.04
1 μM EDTA + 50 nM Fe	5.29±0.22	8.88±0.36	9.59±0.10
Station 84 (42h)			
Control	5.09±0.71	5.42±0.99	6.16±0.41
50 nM Fe	4.54±0.22	5.83±0.46	5.32±0.11
200 nM PO_4-P	8.71±1.00*	10.38±0.69*	9.00±0.48*
50 nM Fe + 200 nM PO_4-P	9.70±1.21*	9.63±0.57*	10.23±0.23*
1 μM EDTA	5.45±0.87	5.21±0.20	5.29±0.48
1 μM EDTA + 50 nM Fe	5.91±0.48	6.43±0.31	5.78±0.36

Note: Values are treatment means (±range/2, $n = 2$) at the end of the stated incubation period. An asterisk indicates that the mean is significantly different from the control ($P = 0.05$).

fractions. Patterns of Fe accumulation into the picoplankton and nanoplankton were consistent among all three stations assayed. Accumulation into the picoplankton was essentially complete at 15–20% of total radioactivity within 20 h, whereas accumulation into the nanoplankton increased slowly (0.44%·h^{-1}) after 20 h (Fig. 2).

The concentration of total dissolved Fe (Fe_T) at Station 935 (34 nM) was approximately twice that measured at Station 23 (12 nM); thus, the specific activity of ^{59}Fe was greater in the Station 23 experiment. However, since the concentration of Chl *a*, a surrogate for planktonic biomass, was approximately twice as great at Station 23 (2.8 μg·L^{-1}) as at Station 935 (1.5 μg·L^{-1}), the ratio of radiolabelled Fe to biomass was essentially the same in both experiments (Station 935: 2.1 kBq·nmol Fe_T^{-1}·μg Chl a^{-1}; Station 23: 2.8 kBq·nmol Fe_T^{-1}·μg Chl a^{-1}).

The observed pattern of non-EDTA-exchangeable ^{59}Fe accumulation is consistent with biological uptake: accumulation into the picoplankton is solely by transport of dissolved Fe species across the cell membrane, whereas accumulation into the nanoplankton is assumed to result from both the internalization of dissolved species and the uptake of ^{59}Fe-containing particles by the phagocytotic activity of nanoplanktonic protozoa, as observed for other trace metals in Lake Erie surface waters (see Twiss et al. 1996).

Discussion

Planktonic Fe demand in pelagic surface waters of Lake Erie

The results obtained in 1996 showed a dramatic increase in growth in the nanoplankton and picoplankton size fractions, whereas those obtained in subsequent years revealed that P alone increased phytoplankton productivity following enrichment. Testing for Fe limitation in the Laurentian Great Lakes is not novel. The addition of chelated Fe (FeEDTA) to surface water collected from southern Lake Huron during the summer months caused a significant increase in phytoplankton (Lin and Schelske 1981), and the addition of high concentrations of unchelated Fe was found to occasionally enhance photosynthesis in plankton collected from the eastern basin of Lake Erie (Storch and Dunham 1986). However, these earlier studies are compromised by the lack of a trace metal clean protocol for collecting and manipulating water samples. It is now widely accepted that rigorous attention to trace metal hygiene is a critical component of protocols designed to manipulate natural water samples for studying phytoplankton interactions with trace metals (Fitzwater et al. 1982). Moreover, the sole use of Fe chelated by a synthetic organic ligand (e.g., EDTA; Lin and Schelske 1981) in natural waters might result, through displacement, in the complexation of toxic trace metals, rendering interpretation of these experiments difficult. Recent work on Fe chemistry in marine waters has shown that iron oxides are maintained in an amorphous and bioavailable state by a rapid photoreduction–oxidation cycle (Wells et al. 1991*a*, 1991*b*). Given the similar elevated pH (≥8) and low photosynthetically active radiation extinction coefficients ($\varepsilon = -0.19 \cdot m^{-1}$) common to epipelagic Lake Erie and midoceanic areas, we enriched our samples with reduced Fe to favor the formation of complexed Fe or amorphous iron oxides which are bioavailable to phytoplankton. Accordingly, our investigation

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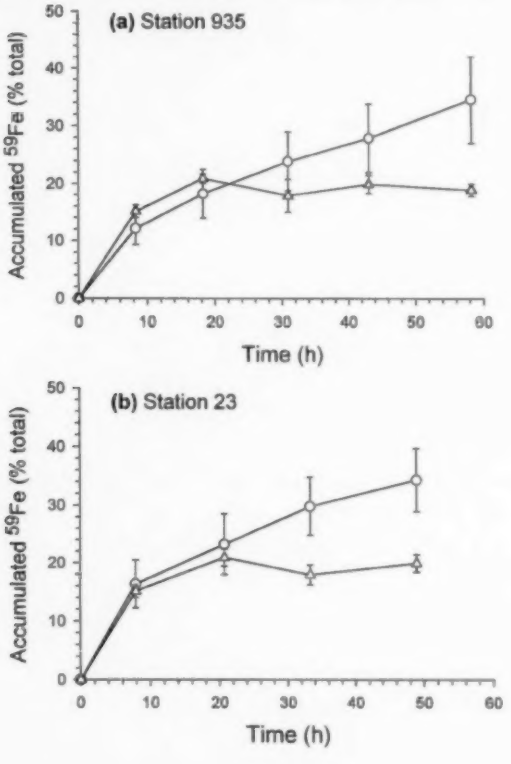
Fig. 2. Accumulation of Fe into the nanoplankton- (○) and picoplankton-sized (△) particle fractions sampled from (*a*) Station 935 and (*b*) Station 23 in the eastern basin of Lake Erie in July 1997. Particles were rinsed with 10 µM EDTA (pH 8) to remove extractable Fe from particle surfaces. Accumulation of ^{59}Fe into nanoplankton was 31.5 ± 6.0% and into picoplankton was 20.7 ± 4.8% after 25 h at Station 953. Values are mean ± SD, n = 3.



utilized trace metal clean protocols and included unchelated Fe additions in order to test the response of the ambient phytoplankton population with minimal disturbance.

Plankton size fractions responded differently to Fe additions. In 1996, the nanoplankton appeared to require much less Fe than the picoplankton. Since the specific growth rates (mean ± SD, per day) of the picoplankton (0.61 ± 0.42) and nanoplankton (0.43 ± 0.26) are similar under these conditions (Twiss and Campbell 1998), it is possible that the nanoplankton required less Fe than the picoplankton. Lower Fe requirements may be due to a lower physiological requirement by eukaryotic phytoplankton (Brand 1991). Alternatively, nanoplanktonic mixotrophic organisms can accumulate Fe through grazing of picoplankton. Some heterotrophic marine protozoans have been shown to satisfy their Fe requirements through phagocytosis (Chase and Price 1997; Maranger et al. 1998). The trophic transfer of bioactive trace metals through the microbial food web (picoplankton → nanoplankton) has been demonstrated for ^{65}Zn and ^{109}Cd in Lake Erie (Twiss et al. 1996) and for ^{59}Fe in the equatorial Pacific (Hutchins et al. 1993). Moreover, the observed pattern of ^{59}Fe accumulation in the picoplankton and nanoplankton observed in the present study (Fig. 2) suggests that organisms in the nanoplankton size-class were accumulating Fe by grazing on picoplankton.

The concentration of bioavailable Fe may have influenced the magnitude of the size-dependent response of the phytoplankton to Fe additions. The rapid response of the picoplankton in 1996, of which cyanobacteria are a significant component, to the Fe addition is consistent with the enhancement of cellular uptake mechanisms in cyanobacteria due to Fe limitation. The ambient chemical conditions in pelagic Lake Erie surface waters during thermal stratification suggest that much of the dissolved Fe is not biologically available, primarily due to complexation by organic ligands (Table 1). Indeed, the values for Fe^{3+} availability (pFe 20.5–21.9) estimated by us for these surface waters indicates a status of Fe limitation in cyanobacteria that corresponds to the induction of high-affinity Fe transport systems (Wilhelm 1995). Our calculations of pFe omit possible enhancements due to in situ photoreduction or remineralization of colloidal iron oxyhydroxides by protozoan grazing activity (Barbeau et al. 1996); however, the effects of these mechanisms on the concentration of dissolved inorganic Fe in the natural environment are not yet assessable.

During the period of thermal stratification, the Lake Erie plankton community may experience a range of Fe nutritive statuses, such as those reported by Hutchins et al. (1998) for the upwelling regions of coastal California. Like epipelagic Lake Erie, this area displays a heterogenous distribution of total dissolved Fe and an even greater order of magnitude range in total dissolved Fe concentrations (0.1–10 nM) that reflect inputs from Fe-depleted oceanic waters and terrestrial influences. According to the multifaceted investigative approach employed by these researchers, the response of the coastal marine phytoplankton community to Fe additions ranged from the subtle to the readily apparent — the response was dependent on the status of the plankton community within the continuum of the Fe limitation, which is represented by four classes (Fe replete, Fe stressed, moderately Fe limited, and severely Fe limited; Hutchins et al. 1998). According to the Hutchins et al. (1998) scheme, the plankton community observed in our experiments would be considered to be at least Fe stressed. Fe additions clearly had a stimulatory effect in 1996, whereas the effects of Fe additions in 1997 and 1998 were subtle, since the plankton had an observeable demand for Fe, as evidenced by the uptake of ^{59}Fe, colimitation with P was observed at some stations, and plankton (>64 µm) sampled during our visit to our research stations exhibited ferredoxin/flavodoxin ratios indicative of Fe stress (R.M. McKay, Bowling Green State University, Bowling Green, Ohio, unpublished data). The phytoplankton community in Lake Erie is most likely physiologically adapted for dealing with transient periods of Fe limitation.

Possible hydrodynamic influences on Fe bioavailability in surface waters

The lack of a clear response to Fe enrichment in 1997 and 1998 could be due to higher epilimnetic Fe concentrations or reduced biological Fe demand. At Station 23, the concentration of total dissolved Fe in 1997 (11.8 nM) was slightly lower than that measured in 1996 (16.2 nM). However, we

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do not have an analytical determination of labile Fe concentration, so it is possible that Fe bioavailability was greater in 1997, despite the lower total dissolved Fe observed at this station in 1996. Instead, a reduced biological Fe demand in 1997 and 1998 may be related to increased turbulent mixing and entrainment of metalimnetic Fe-rich water a few weeks prior to our experiments.

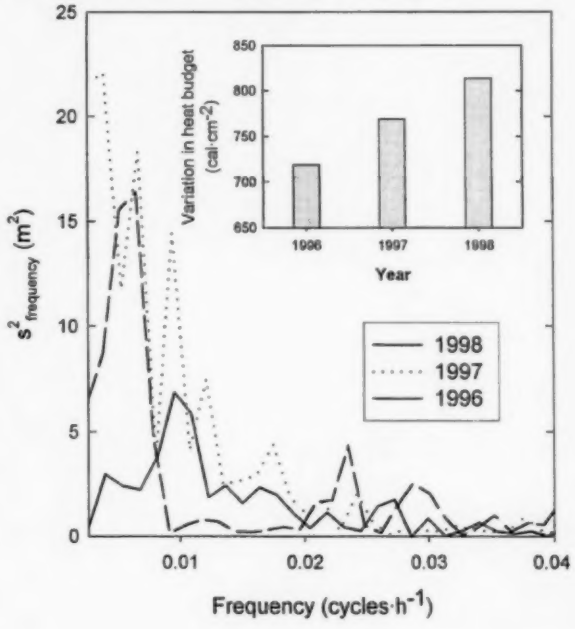
To estimate turbulent mixing from wind-induced surface waves, we obtained hourly records of wave height data from Weather Buoy 45142 (42.7°N, 79.3°W) located in the vicinity of Station 23. Focusing on the 30-day precruise period in each year, the wave height data were decomposed into their dominant frequencies using harmonic analysis (Fig. 3) calculated following the method of Legendre and Legendre (1979). In 1997 and 1998, low-frequency (i.e., unique, <0.01 cycle·h^{-1}, >4-day period) large-amplitude mixing events accounted for 68 and 60% of the total variance in the periodogram, respectively, whereas the amplitude contributions at these frequencies were much lower, representing only 36% of the total variance of the 1996 data set. This analysis clearly demonstrates that intense turbulent mixing characterized the 30-day precruise period in 1997 and 1998, in contrast with 1996 when wind-induced vertical mixing was less intense prior to the research cruise. This interpretation is also supported by the higher differential heat gain by the water column over the 30-day period observed in 1997 and 1998 relative to 1996 (Fig. 3, inset). Therefore, precruise conditioning of the epilimnion through metalimnetic entrainment of Fe-rich water may have alleviated Fe limitation in the surface water phytoplankton community in 1997 and 1998.

We can only speculate that Fe speciation in 1997 may have been markedly different from that existing at this station in 1996 at the same time of year and that Fe bioavailability was greater in 1997 and 1998, leading to a reduced Fe demand by the phytoplankton in these two years. The fact that the plankton were actively accumulating Fe (Fig. 2) illustrates the physiological adaptations of the plankton to assimilate as much of this element as possible when it is available. In support of this proposed adaptative response mechanism of pelagic Lake Erie phytoplankton, phytoplankton in high-nutrient low-chlorophyll regions of the Pacific have been shown to produce Fe-binding ligands for facilitating Fe uptake even when Fe availability was artificially enhanced by a large-scale in situ enrichment (Rue and Bruland 1997). It is possible that phytoplankton in a chemical environment such as the thermally stratified epilimnion of the pelagic Great Lakes (pH ≥8, 2–4 mg DOC·L^{-1}) have similar physiological adaptations that enable them to accumulate this essential element whenever possible. It remains to be seen, however, how often and widespread is the occurrence of plankton growth outstripping the bioavailability of Fe in the surface waters of this and other Great Lakes.

Conclusions

This is the first direct demonstration of trace metal limitation of phytoplankton in the Laurentian Great Lakes using trace metal clean protocols, although we did not observe the phenomenon to be widespread in Lake Erie. Iron alone greatly stimulated phytoplankton growth in 1996, wheras P addition alone stimulated growth in 1997 and 1998. Phosphorus availability is commonly considered the single limiting nutrient for phytoplankton productivity in Lake Erie (Makarewicz 1993), yet Fe bioavailability may be a significant colimiting nutrient during certain periods. Occurrences of Fe limitation in this shallow lake might be related to the time scales of hydrodynamic forcing. Thus, the effect of hydrodynamics on trace metal water chemistry must be investigated with respect to its effect on primary production, shifts in phytoplankton species composition, and heterotrophic plankton productivity.

Fig. 3. Periodogram of wave heights obtained at hourly intervals from Weather Buoy 45142 (42.7°N, 79.3°W) over the 30-day (n = 720) precruise period in 1996, 1997, and 1998. The inset shows the differential heat gain determined from thermal profiles at Station 23 over the same period (1 cal = 4.1868 J).



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References

Barbeau, K., Moffett, J.W., Caron, D.A., Croot, P.L., and Erdner, D.L. 1996. Role of protozoan grazing in relieving iron limitation of phytoplankton. Nature (Lond.), **380**: 61–64.

Beaubien, S., Nriagu, J., Blowes, D., and Lawson, G. 1994. Chromium speciation and distribution in the Great Lakes. Environ. Sci. Technol. **28**: 730–736.

© 2000 NRC Canada

Brand, L.E. 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. Limnol. Oceanogr. **36**: 1756–1771.

Bruland, K.W., Orians, K.J., and Cowen, J.P. 1994. Reactive trace metals in the stratified central North Pacific. Geochim. Cosmochim. Acta, **58**: 3171–3182.

Buffle, J. 1988. Complexation reactions in aquatic systems. Ellis Horwood, Chichester, U.K.

Chase, Z., and Price, N.M. 1997. Metabolic consequences of iron deficiency in heterotrophic marine protozoa. Limnol. Oceanogr. **42**: 1673–1684.

Cheam, V., Lechner, J., Desrosiers, R., Sekerka, I., Lawson, G., and Mudroch, A. 1995. Dissolved and total thallium in Great Lakes water. J. Gt. Lakes Res. **21**: 384–394.

Coale, K.H., and Flegal, A.R. 1989. Copper, zinc, cadmium and lead in surface waters of Lakes Erie and Ontario. Sci. Total Environ. **87/88**: 297–304.

Coale, K.H., et al. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature (Lond.), **383**: 495–501.

Fitzwater, S.E., Knauer, G.A., and Martin, J.H. 1982. Metal contamination and its effect on primary production measurements. Limnol. Oceanogr. **27**: 544–551.

Hudson, R.J., and Morel, F.M.M. 1989. Distinguishing between extra- and intracellular iron on marine phytoplankton. Limnol. Oceanogr. **34**: 1113–1120.

Hutchins, D.A., DiTullio, G.R., and Bruland, K.W. 1993. Iron recycling and regenerated production: evidence for biological iron recycling in two marine environments. Limnol. Oceanogr. **38**: 1242–1255.

Hutchins, D.A., DiTullio, G.R., Zhang, Y., and Bruland, K.W. 1998. An iron limitation mosaic in the California upwelling regime. Limnol. Oceanogr. **43**: 1037–1054.

Legendre, L., and Legendre, P. 1979. Ecologie numérique 2. La structure des données écologiques. Masson/PUQ, Paris.

Lin, C.K., and Schelske, C.L. 1981. Seasonal variation of potential nutrient limitation to chlorophyll production in southern Lake Huron. Can. J. Fish. Aquat. Sci. **38**: 1–9.

Makarewicz, J.C. 1993. Phytoplankton biomass and species composition in Lake Erie, 1970 to 1987. J. Gt. Lakes Res. **19**: 258–274.

Maranger, R., Bird, D.F., and Price, N.M. 1998. Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. Nature (Lond.), **396**: 248–251.

Martin, J.H., and Fitzwater, S.E. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature (Lond.), **331**: 341–343.

Martin, J.H., and Gordon, R.M. 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. Deep-Sea Res. **35**: 177–196.

Martin, J.H., et al. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature (Lond.), **371**: 123–129.

Morel, F.M.M., and Hering, J.G. 1993. Principles and applications of aquatic chemistry. John Wiley & Sons, New York.

Nriagu, J.O., Lawson, G., Wong, H.K.T., and Azcue, J.M. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters. J. Gt. Lakes Res. **19**: 175–182.

Nriagu, J.O., Lawson, G., Wong, H.K.T., and Cheam, V. 1996. Dissolved trace metals in Lakes Superior, Erie, and Ontario. Environ. Sci. Technol. **30**: 178–187.

Raven, J.A. 1988. The iron and molybdenum use efficiencies of plant growth with different energy carbon and nitrogen sources. New Phytol. **109**: 279–287.

Rich, H.W., and Morel, F.M.M. 1990. Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. **35**: 652–662.

Rockwell, D.C., Salisbury, D.K., and Lesht, B.M. 1989. Water quality in the middle Great Lakes: results of the 1985 USEPA survey of Lakes Erie, Huron and Michigan. EPA-905/6-89-001. U.S. Environmental Protection Agency Great Lakes National Program, Chicago, Ill.

Rue, E.L., and Bruland, K.W. 1995. Complexation of iron(III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. **50**: 117–138.

Rue, E.L., and Bruland, K.W. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnol. Oceanogr. **42**: 901–910.

Schenck, R.C., Tessier, A., and Campbell, P.G.C. 1988. The effect of pH on iron and manganese uptake by a green alga. Limnol. Oceanogr. **33**: 538–550.

Storch, T.A., and Dunham, V.L. 1986. Iron-mediated changes in the growth of Lake Erie phytoplankton and axenic algal cultures. J. Phycol. **22**: 109–117.

Tipping, E. 1994. WHAM — a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Comp. Geosci. **20**: 973–1023.

Twiss, M.R., and Campbell, P.G.C. 1998. Scavenging of ^{137}Cs, ^{109}Cd, ^{65}Zn, and ^{153}Gd by plankton of the microbial food web in pelagic Lake Erie surface waters. J. Gt. Lakes Res. **24**: 776–790.

Twiss, M.R., Campbell, P.G.C., and Auclair, J.-C. 1996. Regeneration, recycling and trophic transfer of trace metals by microbial food web organisms in the pelagic surface waters of Lake Erie. Limnol. Oceanogr. **41**: 1425–1437.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. **39**: 1985–1992.

Wells, M.L., Mayer, L.M., Donard, O.F.X., de Souza Sierra, M.M., and Ackelson, S.G. 1991*a*. The photolysis of colloidal iron in the oceans. Nature (Lond.), **353**: 248–250.

Wells, M.L., Mayer, L.M., and Guillard, R.R.L. 1991*b*. A chemical method for estimating the availability of iron to phytoplankton in seawater. Mar. Chem. **33**: 23–40.

Wilhelm, S.W. 1995. Ecology of iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. Aquat. Microb. Ecol. **9**: 295–303.

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Significance and fate of bacterial production in oligotrophic lakes in British Columbia

Thomas Weisse and Erland MacIsaac

Abstract: We measured bacterial abundance, biomass, production rates, and grazing losses to protozoans in three oligotrophic British Columbia lakes and analyzed bacteria abundance and lake productivity data from 11 other lakes, most fertilized with inorganic nutrients to increase their productivity for juvenile salmon. Bacteria contributed about 24% to the phytoplankton–bacteria carbon biomass in the most ultraoligotrophic lakes, and their relative contribution declined to <11% with increasing lake productivity. At increasingly high nutrient loadings to the lakes, bacteria abundance increased and was closely correlated with phytoplankton biomass and productivity. Heterotrophic nanoflagellate (HNF) abundance was positively correlated with bacterial numbers. Grazing experiments revealed that HNF were the primary pathway for moving bacterial production to higher trophic levels in oligotrophic British Columbia lakes, and predation by the ciliate and rotifer microzooplankton community appeared to exert top-down control over the abundance of HNF and the transfer of carbon from bacteria. The HNF and microzooplankton, in turn, were affected by the abundance of crustacean mesozooplankton, principally copepods in our experiments.

Résumé : Nous avons déterminé l'abondance, la biomasse et la production bactériennes, de même que les pertes bactériennes par le broutage des protozoaires, dans trois lacs oligotrophes de la Colombie-Britannique, et analysé des données sur l'abondance bactérienne et la productivité lacustre obtenues pour onze autres lacs dont la plupart avaient été fertilisés par des matières nutritives inorganiques afin d'en accroître la productivité pour les saumons juvéniles. Les bactéries représentaient 24% environ de la biomasse carbonée phytoplancton–bactéries des lacs les plus ultra-oligotrophes et leur apport relatif chutait à <11% à mesure qu'augmentait la productivité des lacs. Aux charges en matières nutritives plus élevées, l'abondance des bactéries augmentait et présentait une corrélation étroite avec la biomasse et la productivité du phytoplancton. L'abondance des nanoflagellés hétérotrophes (NFH) présentait une corrélation positive avec le nombre de bactéries. Des essais de broutage ont montré que les NFH constituaient la principale voie de transformation de la production bactérienne vers les niveaux trophiques supérieurs dans les lacs oligotrophes de la Colombie-Britannique et que la prédation par les ciliés et les rotifères de la communauté microzooplanctonique semblait limiter, du haut vers le bas, l'abondance des NFH et le transfert du carbone à partir des bactéries. Les NFH et le microzooplancton étaient, à leur tour, affectés par l'abondance des crustacés mésozooplanctoniques, surtout les copépodes dans le cas de nos expériences.

[Traduit par la Rédaction]

Introduction

The oligotrophic lakes of British Columbia are among the least productive freshwaters in the world and they are highly sensitive to changes in nutrient loading (Stockner and MacIsaac 1996). Despite their nutrient-poor condition, populations of juvenile sockeye salmon (*Oncorhynchus nerka*) and other planktivorous fish can effectively utilize the limited zooplankton forage of the lakes for growth (Hyatt and Stockner 1985). The carrying capacity of the lakes for planktivorous fish is thought to be limited by both the low productivity of the lakes and the structure of their food webs, and the high densities of planktivores crop down the crustacean zooplankton communities and particularly the cladocerans (Hyatt and Stockner 1985; O'Neill and Hyatt 1987). However, relatively little is known about the food web structures and processes of these lakes other than the "classical food web" components based on the phytoplankton and the mesozooplankton (Stockner and MacIsaac 1996).

Over the last decade, studies have shown that microbial food webs composed of bacteria, autotrophic picoplankton, protozoans, and microzooplankton may be responsible for more of the carbon and energy production and transfer in the pelagic zone of many lakes than the classical phytoplankton–mesozooplankton food chains (Berman 1990; Weisse et al. 1990; Weisse 2000). Indeed, studies in oligotrophic British Columbia lakes have suggested that microbial communities dominate many of the production processes within their plankton communities (Stockner and Shortreed 1989).

As one component of the microbial community, bacterio-

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Table 1. Morphometry, physical and chemical characteristics, and productivity parameters for the three bacteria production and protozoan grazing study lakes.

Parameter	Chilko	Shuswap	Georgie
Area (km^2)	185	310	16
Mean depth (m)	123	62	21
Maximum depth (m)	331	171	40
Mean epilimnetic temperature (°C)	7.5	15.8	14.1
pH	6.9	6.9	6.2
Total dissolved solids ($mg \cdot L^{-1}$)	33	46	18
TP ($mg \cdot m^{-3}$)	2.4	3.5	3.6
Total Chl *a* ($mg \cdot m^{-3}$)	0.80	1.92	0.56

Note: Water quality data are seasonal (May–September) epilimnetic means.

plankton can play an important role in energy and material transfers and recycling within plankton communities (Cole et al. 1988; Simon et al. 1992). However, the pathways for transfer of bacterial production within pelagic food webs are poorly understood. There is debate about the pelagic processes that limit the growth of bacterioplankton and whether bacterial production is efficiently transferred to higher trophic levels (Billen et al. 1990; Thingstad et al. 1997). Many microbial processes are also thought to vary in relative importance (along with other pelagic food web processes) depending on the overall productivity of the waters. Microbial productivity in oligotrophic waters should, according to recent research, reflect a community where bacterioplankton and microbial food webs carry a larger share of the overall production and energy processes of the pelagic community than in more productive lakes (Cole and Caraco 1993).

The primary objective of this study was to determine the magnitude and fate of bacterial production in three oligotrophic British Columbia lakes to understand how pelagic microbial food webs interface with the classical food webs and support planktivorous fish. We made simultaneous measurements of bacterial production and bacteria grazing loss rates by heterotrophic nanoflagellates (HNF) in each of the lakes. To investigate how bacteria were linked to other components of the pelagic food web and the productivity of the lakes, we also analyzed historical bacterial abundance data from 11 lakes relative to changes in total phosphorus (TP) concentrations, phytoplankton biomass (chlorophyll *a* (Chl *a*)), and primary production. This data set was collected during whole-lake nutrient enrichment projects designed to enhance the productivity of a number of oligotrophic sockeye salmon nursery lakes (Stockner and MacIsaac 1996).

Methods

Description of study lakes and data sources

Three different oligotrophic lakes representing a range of lake types in British Columbia were chosen for detailed bacterial production and protozoan feeding experiments. Shuswap Lake is a large (310 km^2), multiarmed, clear, warmwater lake similar to other large lakes in the central interior plateaus of British Columbia. Seasonal TP and Chl *a* levels average 3.5 and 1.92 $mg \cdot m^{-3}$, respectively, and the average epilimnetic temperature is warm at 15.8°C (Table 1). In contrast, Chilko Lake is a large (185 km^2), glacially turbid lake located just east of the coast mountain range. It has a colder thermal structure, and summer inputs of cold, glacial meltwaters keep surface water temperatures relatively low (mean 7.5°C), restricting thermal stratification to July–September. TP and Chl *a* levels in Chilko Lake were roughly half those of Shuswap Lake. Georgie Lake is a small, oligotrophic lake at the northern end of Vancouver Island and it is typical of the many naturally acidic, dystrophic, soft-water lakes of coastal British Columbia. Chl *a* and TP levels averaged 3.6 and 0.56 $mg \cdot m^{-3}$, respectively.

To analyze changes in bacteria abundance relative to changes in other lake productivity parameters, we selected published data for 11 oligotrophic lakes sampled from 1979 to 1984 by the British Columbia Lake Enrichment Program of the Canadian Department of Fisheries and Oceans (MacIsaac et al. 1981; Costella et al. 1982, 1983*a*, 1983*b*; Nidle et al. 1984; Nidle and Shortreed 1985). The lakes are described in detail in the above publications. All of the lakes were coastal, warm, monomictic systems with water clarities ranging from clear to dystrophic, and they all serve as nursery lakes for juvenile sockeye salmon. Nine of the lakes were fertilized weekly through their growing seasons for 1 or more years with inorganic nitrogen and phosphorus nutrients to enhance lake productivity and the growth of sockeye salmon. Areal phosphorus loading rates ranged from about 44 to 370 $mg \cdot m^{-2} \cdot year^{-1}$ at nitrogen to phosphorus atomic ratios of 15:1 to 35:1 (Stockner and MacIsaac 1996). The data set includes unfertilized control lake data and data for fertilized lakes treated over several years at different nutrient loading levels. We selected lakes with a control year (pre- or post-fertilization year), a nearby similar control lake, or a separate unfertilized arm of the same lake.

The lakes were sampled at least monthly through each growing season, and bacteria densities were determined using the acridine orange direct count method using identical techniques for all years (MacIsaac et al. 1981). Methods of TP and Chl *a* analyses are given in Stephens and Brandstaetter (1983). TP data prior to 1980 were not used because of a significant change in analytical method. Primary production measurements (hourly mean volumetric carbon uptakes) were only made at selected lakes using sodium bicarbonate ^{14}C techniques, in situ midday 2- to 4-h incubations, and dissolved inorganic carbon concentrations calculated from pH and alklinity determinations (Costella et al. 1982). Annual growing season (May–September inclusive) epilimnetic means were calculated for each lake from depth profiles of bacteria densities, TP, total Chl *a*, and primary production. Where multiple sampling stations were present in each lake, data were pooled.

Bacterial production and grazing experiments

Bacterial production and protozoan grazing studies in Georgie and Chilko lakes and the Main Arm of Shuswap Lake were conducted using mixed epilimnetic water from midlake stations. Georgie and Shuswap lakes were sampled twice each, in the spring and summer, while Chilko Lake was only sampled once in July after thermal stratification was well developed. Samples were collected with a 6-L Van Dorn water bottle from several depths within the epilimnion and pooled.

HNF and bacterioplankton were counted and sized using epifluorescence microscopy and DAPI staining (Porter and Feig 1980). Samples were fixed with 2% filtered (0.2 μm), buffered formaldehyde and stored cold, and 3- to 4-mL replicate subsamples for bacteria counts were stained with DAPI, filtered onto Irgalan-Black-dyed 0.2-μm, 25-mm-diameter polycarbonate filters (Nuclepore), rinsed, and enumerated at 1250× magnification with a Zeiss epifluorescence microscope equipped with an UV excitation filter set. Average bacteria cell volumes were determined using black and white slide photomicrographs taken of replicate fields for each subsample. Cell dimensions were measured from the inner bright edge of the cell for 25–40 bacteria per field by projecting the slides and calibrating the measurements using a sizing

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graticule. Individual cell volumes were calculated using geometric formulae (Fuhrman 1981). Bacterial carbon content was calculated from measured volumes using an allometric relationship between cell size and biovolume (Simon and Azam 1989). For data from previous years where bacterial volumes had not been measured, we assumed a carbon content of 20 fg·cell^{-1} (Lee and Fuhrman 1987).

Bacterial production rates were determined by measuring L-[4,5-^{3}H]leucine incorporation into bacterial protein (Kirchman 1993). Replicate to quadruplicate 20-mL samples received inoculations of radiolabelled leucine to 10 nM final concentrations and were incubated for 0.5–1 h at in situ temperatures. Blanks received 2 mL of 50% trichloroacetic acid (TCA) before inoculation. After incubation, samples were killed with 2-mL additions of 50% TCA and filtered onto 0.22-μm cellulose acetate filters that had been presoaked in 0.4 mM leucine. Filters were rinsed three times with 3-mL additions of ice-cold 5% TCA and then rinsed twice with 2-mL additions of ice-cold 80% ethanol. Filters were placed in scintillation vials, air dried, and dissolved with 0.5 mL of ethyl acetate. Scintillation cocktail (10 mL, Scintiverse II, Fisher Scientific) was added and samples were radioassayed with a Packard model 460 scintillation counter. Counts were quench corrected using a custom tritium quench series constructed using the same filters and scintillation cocktail as for the samples. Bacterial production was calculated using an empirically determined conversion factor of 2.23×10^{17} cells·mol leucine incorporated^{-1}. Calibrations were made in Shuswap Lake in April 1994 by diluting 1.0-μm-filtered sample water with 0.2-μm-filtered sample water and incubating and sampling at intervals over 72 h for leucine incorporation and bacteria abundance. The carbon conversion factor was calculated using the "modified derivative method" (Kirchman and Ducklow 1993). We repeated the calibration series in Georgie Lake in August 1994. Because the conversion factor was close to the one obtained in Shuswap Lake in spring, we used the former factor for all cases.

Grazing rates on bacteria by protozoans were determined by measuring the rate of incorporation of heat-killed, [^{3}H]leucine-radiolabelled bacteria into the >1.0 μm size fraction (Weisse 1999). For grazing experiments, the individual water samples were combined to give one integrated sample of epilimnetic water. To label the bacteria, 500 mL of lake water from each sampling site was gravity filtered through several 1.0-μm, 47-mm-diameter Nuclepore polycarbonate filters, and 6–10 mL of the radioactive [^{3}H]leucine solution (specific activity 158–171 Ci·mmol^{-1} (1 Ci = 37 GBq), diluted 1:30 with 0.4 mM nonradioactive leucine) was added. Bacteria were labelled at the in situ temperature for 24 h. The suspension was then filtered on several 0.2-μm, 47-mm-diameter Nuclepore filters, and labelled bacteria were resuspended by rinsing the filters several times with 0.2-μm-filtered lake water. The volume of this suspension was made up to only 200 mL to compensate for losses of bacteria during filtering and resuspending. We checked the efficiency of this method by measuring cell concentrations before and after filtering and resuspending bacteria. Our procedure of harvesting and washing radiolabelled bacteria resulted in a yield of 20–70% of the initial bacterial concentration. Because bacteria were concentrated during resuspension (500 mL reduced to 200 mL), the final concentration of radiolabelled bacteria was close to the initial concentration of unlabelled bacteria.

The labelled resuspended bacteria were heat killed at 70–80°C for 1 h. The grazing experiments were started after cooling the suspension to lake water temperature. The suspension of radiolabelled heat-killed bacteria was combined with fresh epilimnetic lake water ("grazer" water) in a ratio of 1:2. The experimental water had been prefiltered through 100-μm Nitex mesh to remove large crustacean zooplankton. To prevent living bacteria that were introduced with the grazer water from growing, we added an antibiotic cocktail of penicillin–streptomycin (final concentration 100 units penicillin·mL^{-1} and 100 mg streptomycin·L^{-1}) about 10 min before the start of the experiments. Replicate to quadruplicate subsamples of 20 mL each were taken at the beginning of the experiments and after 1 h. Uptake rates were measured in three different size fractions by sequential filtration through 8.0-, 1.0-, and 0.2-μm Nuclepore filters. The extraction procedure and preparation of filters for scintillation counting were identical to the measurement of bacterial production described above. The ^{3}H specific activity lost in the bacterial (0.2–1 μm) fraction or taken up by the two bacterivore size-classes (1–8 μm HNF, 8–100 μm ciliates and other microzooplankton) was determined as the difference between the final and initial ^{3}H activity of each filter size. Ingestion rates were calculated by dividing the specific activity taken up by the two larger size categories at the end of the experiments by the total initial specific activity of the bacterial assemblage (Caron et al. 1993). Loss rates occurring in the bacterial fraction during the experiments gave an independent estimate of total bacterial grazing loss. Results of bacterial production and grazing measurements reported are arithmetic means. Time series experiments were also conducted to track the movement of ^{3}H activity through the bacterial, flagellate, and microzooplankton size fractions for up to 4 h, but only one experiment from Shuswap Lake in June is reported here.

Samples for Chl *a* determination were filtered onto 0.45-μm Millipore HA filters and frozen until analysis. Chl *a* concentrations were measured fluorometrically after extraction in 90% acetone (Stephens and Brandstaetter 1983). Crustacean zooplankton were collected and densities and species composition were determined from samples collected by vertical hauls with a Wisconsin net (100-μm-mesh Nitex) from the bottom of the epilimnion or from mixed water bottle casts (30 L) throughout the epilimnion by screening through 100-μm Nitex.

Statistical analyses

Changes in the specific radioactivity over time and between different size fractions with and without zooplankton added were tested for significant differences using two-way analysis of variance (ANOVA) under SigmaStat 2.0. If the effect of the factors (time and size fraction or time and zooplankton) and their interaction was significant, pairwise multiple comparison procedures were used (Tukey test) to isolate which group(s) differed. Raw data were logarithmically transformed prior to least-squares linear regression analysis. Differences in bacteria and lake productivity parameters between fertilized and control lakes and years were tested with Student's *t* test after logarithmic transformation. If not stated specifically, significant differences denote $p < 0.05$. Standard deviations (±SD) are given where $n > 1$.

Results

Bacterial abundance, production, and grazing loss rates

The epilimnetic concentrations of bacteria in Shuswap, Chilko, and Georgie lakes during the experiments ranged between 0.38×10^{12} and 0.72×10^{12} bacteria·m^{-3} with lowest mean cell numbers in Chilko Lake ($0.40 \times 10^{12} \pm 0.05 \times 10^{12}$ bacteria·m^{-3}). Bacterial abundance was significantly higher ($p < 0.01$) in Georgie Lake ($0.52 \times 10^{12} \pm 0.06 \times 10^{12}$ bacteria·m^{-3}) than in Chilko Lake. Surveys of bacteria abundance within Shuswap Lake varied from lowest densities in epilimnetic waters of the oligotrophic Main Arm to higher cell numbers (up to 1.8×10^{12} bacteria·m^{-3}) in the more productive Salmon Arm. The average bacterial abundance in Shuswap ($0.89 \times 10^{12} \pm 0.40 \times 10^{12}$ bacteria·m^{-3}) was significantly higher ($p < 0.02$) than in the two more oligotrophic lakes. Maximum cell numbers generally occurred in late summer samples.

The average epilimnetic density of HNF, the primary bacterivores recorded during the experiments, was signifi-

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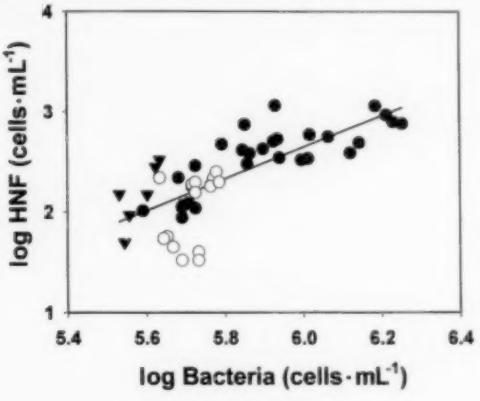
Fig. 1. Least-squares log–log regression of the mean epilimnetic abundance of HNF versus bacteria concentrations in Chilko (triangles), Georgie (open circles), and Shuswap (solid circles) lakes over their growing seasons. The regression was significant: log HNF = 1.58 log bacteria – 6.82 ($r^2 = 0.56$, $n = 50$, $p < 0.01$).



cantly higher ($p < 0.02$) in Shuswap Lake ($445 \times 10^3 \pm 293 \times 10^3$ cells·m^{-3}) than in Chilko ($167 \times 10^3 \pm 100 \times 10^3$ cells·m^{-3}) and Georgie ($133 \times 10^3 \pm 83 \times 10^3$ cells·m^{-3}) lakes. The difference between HNF numbers in the latter two lakes was insignificant. Overall, HNF were significantly correlated with bacterial abundance (Fig. 1). According to the least-squares linear regression equation of the log-transformed data (log HNF = 1.58 log bacteria – 6.82, $r^2 = 0.56$), 56% of the variation in seasonal HNF cell numbers in the three lakes can be explained by bacterial abundance.

Bacterial carbon production measured on seven occasions in the three study lakes is reported in Table 2 together with bacterial cell numbers, biovolumes, and total bacterial carbon biomass. Bacterial cell volumes and carbon biomass were lowest in Chilko Lake in July and highest in Georgie Lake in August. In Shuswap Lake, bacterial cell volumes and carbon were similar between spring and early summer. Except for one value of 3.47 mg C·m^{-3}·h^{-1} measured at one station in Georgie Lake in August, bacterial production varied relatively little from 0.56 to 1.82 mg C·m^{-3}·h^{-1}.

We compared bacterial production (cells produced per millilitre per hour) with grazing loss rates (cells removed per millilitre per hour) caused by nano- and micro-zooplankton (<100 µm) grazing (Fig. 2). On three of five occasions, bacteria production and grazing loss rates appeared to be virtually balanced within the epilimnetic waters of the lakes. Bacteria production rates that were significantly (*t* tests, $p < 0.05$) in excess of bacterial losses were observed in Shuswap Lake in June and Georgie Lake in August. On both sampling occasions, the plankton communities were characterized by relatively high crustacean zooplankton concentrations exceeding 5000 animals·m^{-3}. The mesozooplankton community, however, was not directly responsible for any of the bacterial grazing loss rates reported because larger zooplankton were excluded from our experiments by prescreening through 100-µm-mesh Nitex. Copepods dominated the zooplankton assemblage in both cases, with adult and late-copepodite *Diacyclops thomasi*, *Leptodiaptomus ashlandi*, and *Skistodiaptomus oregonensis* being the numerically dominant species. The combined abundance of these species reached 7700 animals·m^{-3} in Shuswap Lake in June and 5000 animals·m^{-3} in Georgie Lake during the August experiment. The remaining zooplankton species present, although not abundant, were the cladocerans *Bosmina longispina*, *Holopedium gibberum*, and *Daphnia longiremis*.

Size fractionation of the bacterial ^{3}H specific activity during the first hour of the grazing experiments revealed that the major part of the uptake of the bacterial label was by the 1–8 µm size-class, corresponding to the HNF community (Fig. 3). The 8–100 µm size fraction ("ciliate–microzooplankton fraction") was dominated by ciliates during the experiments (enumeration data not shown) and contributed <20% to the overall grazing losses of the bacterial community. Bacteria loss rates ranged from 2.2 to 7.0%·h^{-1}, with the highest percent loss of the bacterial community occuring in Chilko Lake, a lake with one of the lowest bacterial production rates in the study.

In Shuswap Lake in June, we extended the grazing experiments with labelled bacteria for 4 h and added mesozooplankton (>100 µm) concentrated from 30 L to a parallel series of experiments to determine how the crustacean zooplankton community affects nanoflagellate and protozoan grazing on the bacteria community. The relative changes over the course of the incubation are indicated in Fig. 4. The experimental duration and the presence or absence of zooplankton both significantly affected the disintegrations per minute (DPM) levels (two-way ANOVA, $p < 0.05$). Without mesozooplankton present (Fig. 4, top panel), the radioactivity was significantly different (ANOVA, $p < 0.01$) between the different size fractions. DPM levels in the bacterial fraction (0.2–1.0 µm) declined linearly and significantly over the first 3 h (least-squares linear regression, $r^2 = 0.98$). Radioactivity was higher in the HNF fraction (1.0–8.0 µm) 1 h after the beginning of the experiment and then declined. The difference was significant (Tukey test, $p < 0.05$) between values measured at 1 and 4 h after the beginning of the experiment. Uptake rates in the larger ciliate–microzooplankton fraction (8.0–100 µm) began to increase 1 h after the beginning of the experiment, while the initial uptake of the HNF fraction declined, indicating a trophic transfer of label from HNF to the ciliate size-class. At the end of the experiment, mean DPM levels in the HNF fraction were not significantly different from their initial values, while radioactivity in the bacterial fraction was significantly lower and in the microzooplankton fraction was significantly higher than at the beginning (Tukey test for each fraction). The final DPM levels differed significantly (ANOVA) between the three size fractions.

This pattern changed distinctly when we added concentrated mesozooplankton to the experimental containers (Fig. 4, bottom panel). Mesozooplankton were dominated by late-instar cyclopoid and calanoid copepods, and total initial mesozooplankton abundance in this experiment was 8.5 animals·L^{-1}. The HNF fraction showed a larger initial increase in ^{3}H label, while concentrated mesozooplankton clearly prevented the accumulation of bacterial label in the ciliate fraction by the end of the 4-h incubation. The copepods that comprised the bulk of the crustacean zooplankton present suppressed the protozoan, ciliate, and microzooplankton assemblages. Although less significant than in the experiment without zooplankton, the radioactivity was different between the

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Table 2. Bacterial volume, abundance, carbon biomass, and production estimates at lake sampling stations during the bacterial production and grazing experiments.

Lake station and sample season	Mean bacterial cell volume (μm^3)	Bacterial abundance ($10^{12} \cdot m^{-3}$)	Total bacterial carbon ($mg \cdot m^{-3}$)	Bacterial carbon production ($mg \cdot m^{-3} \cdot h^{-1}$)
Shuswap, April	0.139	0.50	13.9	0.56
Shuswap, June	0.133	0.72	19.0	0.90
Georgie, May				
Station A	0.142	0.69	19.6	0.76
Station B	0.159	0.68	21.6	1.25
Georgie, August				
Station A	0.180	0.67	24.1	1.82
Station B	0.198	0.64	25.3	3.47
Chilko, July	0.112	0.38	8.5	0.65

Fig. 2. Comparison of the number of bacteria cells produced (open bars) and the number of cells lost to grazing (solid bars) for epilimnetic waters from Shuswap, Georgie, and Chilko lakes. Error bars denote 1 SD.

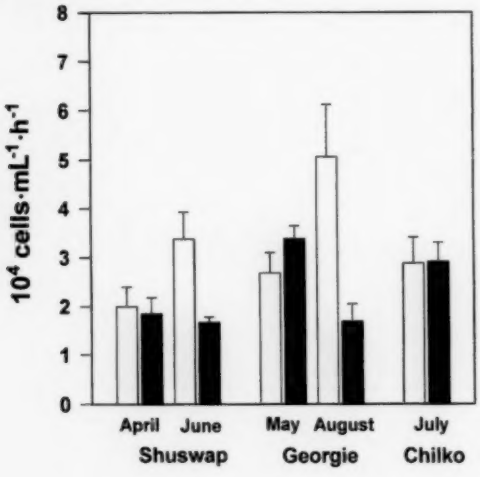
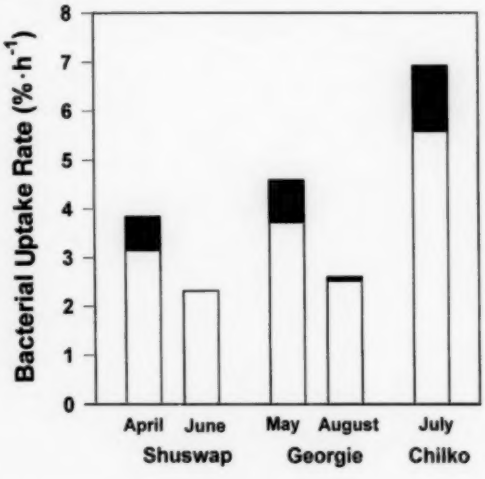


Fig. 3. Bacteria losses to the two grazer size-classes for Shuswap, Georgie, and Chilko lakes. The HNF size-class corresponds to the 1–8 μm size range (open bars), and microzooplankton (ciliates, rotifers) dominate the 8–100 μm size-class (solid bars).



three size fractions both over the course of the experiment ($p = 0.059$) and when only the final values were taken ($p < 0.05$). At the end of the incubation period, the activity in the bacteria fraction (<1.0 μm) was significantly higher than at the beginning, presumably from ^{3}H label recycled by the mesozooplankton back to the bacterial community and a relaxation of top-down grazing control.

Bacterial responses to lake productivity changes

Bacteria densities and lake productivity parameters all increased in response to inorganic nutrient additions in fertilized lakes compared with the control lakes (Table 3). Mean TP concentrations (all lakes and years) more than doubled from 1.4 ± 0.3 to 3.4 ± 1.7 $mg \cdot m^{-3}$. Phytoplankton Chl *a* levels and primary production rates were three- to four-fold higher in the fertilized lakes, increasing from 1.12 ± 0.59 to 3.15 ± 2.32 mg Chl $a \cdot m^{-3}$ and from 0.89 ± 0.44 to 3.67 ± 2.70 mg $C \cdot m^{-3} \cdot h^{-1}$. Bacteria responded to nutrient enrichment with an average doubling in abundance from 0.63 × 10^{12} ± 0.14 × 10^{12} to 1.28 × 10^{12} ± 0.38 × 10^{12} $bacteria \cdot m^{-3}$. All the differences in bacteria and lake productivity parameters between control and fertilized lake mean values were significant (*t* test, $p < 0.01$, log transformed).

Bacteria abundance in the epilimnia of the lakes was significantly correlated with the TP concentrations in the lakes (Fig. 5), explaining 58% of the variation in epilimnetic bacteria (log bacteria = (5.79 ± 0.03) + (0.57 ± 0.08) log TP, $r^2 = 0.58$). Bacteria biomass accounts for relatively little of the TP. At 20 fg $C \cdot cell^{-1}$ and a 106:1 carbon to phosphorus ratio, 10^{12} $bacteria \cdot m^{-3}$ would contribute only 0.2 mg $P \cdot m^{-3}$ to the TP range of 1.5–4.5 $mg \cdot m^{-3}$ recorded in the fertilized lakes.

Epilimnetic bacteria abundance was correlated with both Chl *a* biomass and primary production over the range of phytoplankton productivities in the lakes (Fig. 6). The log–log regressions were significant for both epilimnetic Chl *a* levels (log bacteria = (5.89 ± 0.02) + (0.41 ± 0.05) log Chl *a*,

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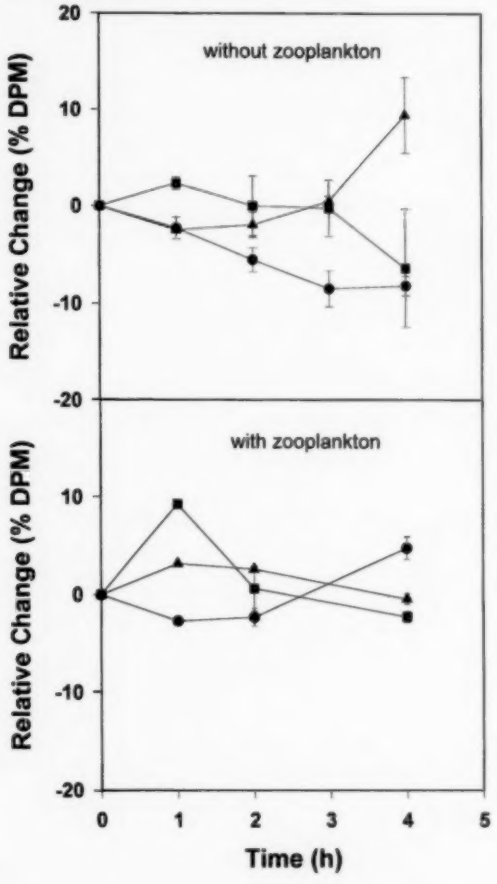
Fig. 4. Time course of ^3H label movement from heat-killed bacteria (0.2–1 µm, circles) into the HNF (1–8 µm, squares) and microzooplankton (8–100 µm, triangles) size fractions in Shuswap Lake in June 1994. The top panel experiment was with zooplankton >100 µm removed, and the bottom panel experiment was with concentrated zooplankton (>100 µm) added to the experimental containers. Error bars denote 1 SD.



$r^2 = 0.55$) and primary production rates (log bacteria = (5.91 ± 0.03) + (0.41 ± 0.06) log primary production, $r^2 = 0.67$), accounting for 55 and 67%, respectively, of the variation in epilimnetic bacteria abundance. The percentage of bacterial carbon biomass to total bacterial plus phytoplankton carbon biomass (20 fg C·bacteria cell^{-1}; 50:1 carbon to Chl *a* ratio) was about 24% at Chl *a* concentrations of 1 mg·m^{-3} and decreased to 11% at a higher Chl *a* concentration of 5 mg·m^{-3}.

Discussion

Ours is the first study assessing the fate of bacterial production in the context of the microbial food web structure in oligotrophic lakes in British Columbia. Because there is at present no standard method available to measure bacterial grazing loss rates (Landry 1994), we will discuss limitations of the approach used in this investigation before considering the ecological implications of the results.

Potential and limitations of bacterial grazing experiments using radiolabelling

Our experimental protocol is identical to that of a recent study conducted in oligotrophic marine waters (Weisse 1999) and similar to the approach of Caron et al. (1993) who used tritiated thymidine to label bacteria. Advantages and limitations of the radioisotope labelling technique have been discussed extensively by these authors and by Landry (1994). The major advantages of radiolabelling are its high analytical sensitivity and short incubation times, the use of natural bacterial assemblages, and the ability to measure bacterivory simultaneously in separate grazer size fractions. Disadvantages originate from the need for enclosure in experimental containers and, in the case of bacteria, alteration of the natural populations during radiolabelling and heat killing. Visual inspection under the microscope gave no indication that the size of bacteria had changed or that bacteria had aggregated during labelling in our experiments. The motility and some surface properties of the bacteria were, however, undoubtedly affected by the labelling and heat-killing procedures. The magnitude of the effect of these potential artifacts on the experimental results remains unknown. One problem with the approach taken is that negative changes in percent DPM levels relative to initial values may result from loss of initial (background) activity. If, for instance, flagellates were strongly reduced by ciliate grazing during the course of the experiment, the background DPM level of the flagellate fraction may have been lower at the end than at the beginning of the experiment (cf. Fig. 4, top panel). Similarly, the initial addition of antibiotics may not prevent heterotrophic bacteria from taking up recycled labelled material if the experimental period exceeds 2–3 h. This is probably due to a combined effect: first, growth-inhibiting antibiotics such as penicillin–streptomycin may not completely stop bacterial uptake of dissolved organic material, and second, the efficiency of the inhibition may decline several hours after the beginning of the experiments (Weisse 1989). In spite of these problems, the technique that we used has been shown to be internally consistent and seems to be robust against experimental manipulations (Weisse 1999). The standard error of the grazing estimates in each size fraction is about 30% and thus comparable with the precision obtained in routine bacterial production measurements (Weisse 1999 and references therein).

The strength of our technique is that it captures short-term dynamics between bacteria and their grazers that were neglected in most recent bacterivore grazing studies (Wickham 1998; Weisse 1999 and references therein). The time series experiments demonstrated the importance of the experimental duration for the interpretation of the results obtained. There seemed to be two phases in the grazing experiments: a first phase when label accumulated in grazing HNF and decreased in the bacteria assemblage and a second when larger protists and mesozooplankton (if present) grazed down the HNF and released bacteria from HNF grazing pressure. The fact that the uptake of label by mesozooplankton responded after a time delay in our experiments may point to an initial

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Table 3. Means (and SD when $n > 1$) of seasonal epilimnetic average bacteria densities, TP concentrations, Chl *a* levels, and primary production rates for fertilized and control (pre- or post-fertilization) years in 11 oligotrophic coastal British Columbia lakes.

Lake	Bacteria density (10^{12}·m^{-3})	TP (mg·m^{-3})	Chl *a* (mg·m^{-3})	Primary production (mg C·m^{-3}·h^{-1})
North and central coastal dystrophic lakes				
Bonilla (53°31′N, 130°15′W)				
Control (1979)	0.77	nd	1.19	1.80
Fertilized (1980–1983)	1.67 (0.42)	5.6 (1.2)	4.98 (0.92)	5.47 (1.26)
Curtis (53°30′N, 129°50′W)				
Fertilized (1980–1983)	1.50 (0.24)	4.6 (1.7)	3.89 (0.78)	3.96 (2.55)
Devon (53°27′N, 129°45′W)				
Fertilized (1980)	1.64	3.2	4.72	2.87
Lowe (53°34′N, 129°33′W)				
Fertilized (1979–1980)	1.21 (0.06)	2.6	1.27 (0.62)	1.37 (0.05)
Long (51°14′N, 127°10′W)				
Control (1980–1981)	0.76 (0.20)	1.7 (0.2)	2.18 (0.41)	0.94 (0.27)
Fertilized (1979, 1982, 1983)	0.99 (0.16)	3.9 (0.8)	3.15 (0.87)	2.45
Vancouver Island clear-water and dystrophic lakes				
Woss (50°08′N, 126°38′W)				
Control (1979, 1982)	0.56 (0.12)	1.0	0.94 (0.18)	1.02
Nimpkish (50°25′N, 126°57′W)				
Fertilized (1982–1984)	0.83 (0.11)	1.6 (0.6)	1.73 (0.93)	nd
Sproat (49°14′N, 125°06′W)				
Control (1981–1984)	0.52 (0.04)	1.2 (0.2)	0.51 (0.12)	0.43 (0.11)
Great Central (49°22′N, 125°15′W)				
Fertilized (1979, 1980, 1982–1984)	0.99 (0.10)	1.7 (0.3)	1.10 (0.21)	0.97 (0.35)
Hobiton (48°45′N, 124°49′W)				
Control (1984)	0.84	2.1	1.30	nd
Fertilized (1980–1983)	1.45 (0.17)	3.2 (0.8)	2.25 (0.36)	nd
Kennedy Main Arm (49°04′N, 125°30′W)				
Control (1981, 1983, 1984)	0.61 (0.03)	1.5 (0.2)	1.25 (0.20)	0.80 (0.04)
Fertilized (1980, 1982)	1.14 (0.64)	1.8 (0.1)	1.64 (0.69)	1.17 (0.74)
Kennedy Clayoquot Arm (49°08′N, 125°35′W)				
Fertilized (1980–1984)	1.44 (0.46)	4.3 (1.1)	5.76 (4.14)	5.85 (3.70)

Note: Fertilized lakes received weekly additions through the growing season of inorganic nitrogen and phosphorus fertilizers. Control data were not available for some lakes, and similar nearby lakes or separate arms of the same lake were used as controls. There are no data (nd) for some parameters.

disturbance due to the experimental manipulation and (or) a stimulus from increased HNF activity during the course of the experiments.

Relationships between bacteria, HNF, and mesozooplankton

The relationship between bacteria and HNF has been controversial in the literature (Berninger et al. 1991; Sanders et al. 1992; Gasol and Vaqué 1993; Gasol et al. 1995). From their literature survey across different ecosystems including sediments, Berninger et al. (1991) suggested a close coupling between bacteria and HNF, with the slope of the log HNF versus log bacterial abundance linear regression close to unity (1.12 ± 0.07, 95% CI) with a fairly constant numerical relationship of about 1000 bacteria per flagellate (B:F). These authors and Sanders et al. (1992) concluded that HNF cell numbers in oligotrophic ecosystems are largely controlled by bottom-up processes, i.e., the abundance of their bacterial food. Gasol and Vaqué (1993), in contrast, reanalyzed the literature data and found only weak coupling between bacteria and HNF over a wide trophic range. The B:F ratio was variable in their study and declined along a gradient from oligotrophic to eutrophic systems. The slope of the log bacteria versus log HNF abundance regression in Gasol and Vaqué's (1993) analysis of 51 lakes was 0.74 ± 0.11 (SD), significantly less than 1. This regression explained, however, only 50% of the observed variance over a wide range of data, and these authors pointed to the significance of predation and, to a lesser extent, temperature for controlling HNF abundance. Gasol et al. (1995) further demonstrated that top-down effects (predation) were as important as food supply for controlling HNF abundance in 16 mostly oligotrophic Quebec lakes. Cladocerans such as *Daphnia* and *Bosmina* had a strong positive impact on the B:F ratio. The slope of the regression between log-transformed HNF and bacterial abundance was 0.70 ± 0.10 (SD) if the Quebec lakes were combined with literature data from 28 other lakes. Sanders et al. (1992) found a slope of 0.90 for the respective relationship in their cross-system comparison. Note that the emerging conclusion is the opposite of the earlier studies (Berninger et al. 1991; Gasol and Vaqué 1993) by the same authors and their co-workers: the

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Fig. 5. The log–log regression of mean epilimnetic bacteria densities in fertilized (solid circles) and control (open circles) British Columbia lakes as a function of mean epilimnetic TP concentrations. The regression was significant: log bacteria = (5.79 ± 0.03) + (0.57 ± 0.08) log TP (r^2 = 0.58, n = 42, p < 0.01). Fertilized lakes received weekly additions of inorganic nitrogen and phosphorus nutrients for the duration of their growing seasons.

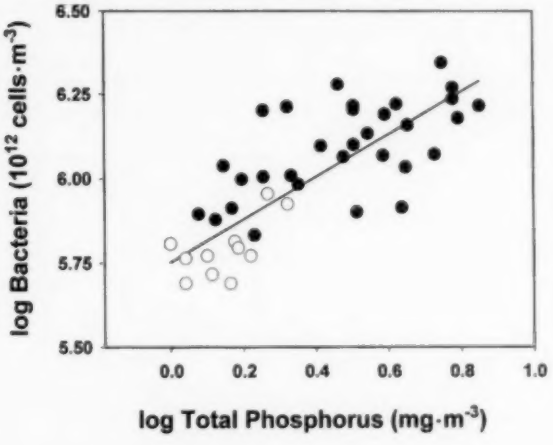
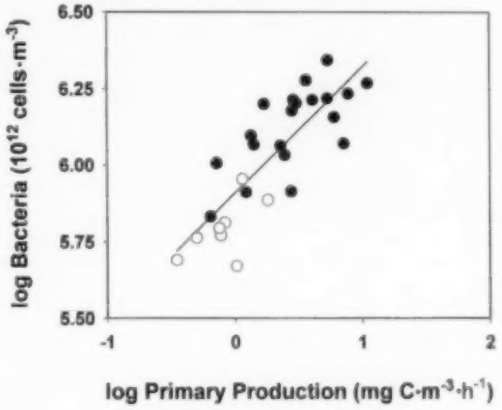
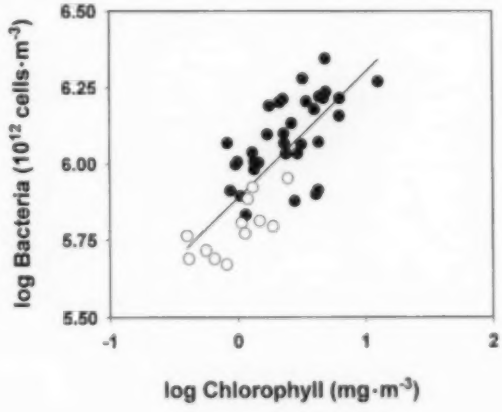


Fig. 6. The log–log regression of mean epilimnetic bacteria densities in fertilized (solid circles) and control (open circles) British Columbia lakes as a function of mean epilimnetic phytoplankton primary production or Chl *a* biomass. The regressions were significant: log bacteria = (5.89 ± 0.02) + (0.41 ± 0.05) log Chl *a* (r^2 = 0.55, n = 47, p < 0.01); log bacteria = (5.91 ± 0.03) + (0.41 ± 0.06) log primary production (r^2 = 0.67, n = 28, p < 0.01). Fertilized lakes received weekly additions of inorganic nitrogen and phosphorus nutrients for the duration of their growing seasons.





B:F ratio increases with bacterial abundance in eutrophic systems. In addition to regional variations, Gasol et al. (1995) also reported considerable temporal changes of the B:F ratio within individual lakes.

Our results are, however, not directly comparable with those of the other studies discussed above, which all used larger data sets over a much wider trophic range. Below a bacterial concentration of approximately 10^{12} cells·m^{-3} in our study, the relative increase in HNF was higher than that of bacteria (Fig. 1). This might imply a bacterial abundance threshold known from other oligotrophic waters (summarized by Weisse 1989) above which HNF numbers rapidly increase and grazing becomes effective.

Our experiments clearly demonstrated the significance of HNF as primary bacterial predators in the oligotrophic British Columbia lakes. There is, however, also some indication (Fig. 1) that HNF levelled off above 10^{12} bacteria·m^{-3}, pointing to the importance of top-down effects on HNF at elevated bacterial concentrations. The significance of mesozooplankton, mainly copepods, as protozoan grazers was obvious from our experiments with concentrated zooplankton. Many copepod genera such as *Skistodiaptomus*, *Diacyclops*, and *Cyclops* are known to feed raptorially in the HNF and microzooplankton size-classes (Lane 1978; Wickham 1995, 1998). We could not include cladocerans and copepods in our statistical analysis because zooplankton data were unavailable for most sampling dates. The zooplankton assemblages enumerated during the grazing experiments suggest that cladocerans were of minor importance in Shuswap, Georgie, and Chilko lakes at the time of the studies. Resident populations of juvenile sockeye salmon are effective planktivores in these British Columbia lakes, capable of cropping down the mesozooplankton communities and particularly the cladocerans (O'Neill and Hyatt 1987). The experiments without mesozooplankton revealed that microzooplankton (mainly ciliates) were the major HNF predators in our study, which confirms recent results from other lakes and oligotrophic marine areas (summarized in Weisse 2000). We conclude that at these low abundance levels, the impact of mesozooplankton predation on HNF may be weak in these oligotrophic British Columbia lakes.

Bacterial response to lake productivity changes

With seasonal mean TP concentrations of 1.0–5.6 mg·m^{-3} and Chl *a* concentrations between 0.51 and 5.7 mg·m^{-3}, our study lakes clearly fall into the category of oligotrophic and ultraoligotrophic lakes (Forsberg and Ryding 1980) even after fertilization with inorganic nitrogen and phosphorus

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(Table 3). The same conclusion generally holds true for bacterial abundances (0.52×10^{12} to 1.67×10^{12} bacteria·m^{-3}) because according to an empirical relationship between Chl *a* and bacterial numbers, oligotrophic waters should average less than 1.7×10^{12} bacteria·m^{-3} (Bird and Kalff 1984).

Mean seasonal TP level is a good surrogate parameter for the nutrient load to these oligotrophic lakes (Stockner and MacIsaac 1996). Much of the variation in bacteria and lake productivity responses within and among lakes reflects year-to-year and between-lake differences in fertilization loading rates prescribed by the Department of Fisheries and Oceans Lake Enrichment Program. The magnitude of nutrient loadings to the Vancouver Island lakes was generally lower than to the north coastal lakes due to the much larger surface area and epilimnia of the former, and the magnitude of changes in bacteria and lake productivity generally reflect that. In the regression relationships between bacteria and the nutrient and lake productivity parameters, the control lake data were typically clustered with the exception of two control year data points from unfertilized years immediately following fertilized years (Hobiton Lake, 1984; Long Lake, 1980) (Figs. 5 and 6). Although the two lakes have water residence times of about 1 year, the suggestion is that some carryover of nutrients and plankton productivity occurs from year to year.

The slope of the log bacteria versus log TP regression was 0.57 ± 0.08 for the British Columbia lakes compared with the slope of 0.91 obtained by Shortreed and Stockner (1986) for Yukon Territory lakes pooled with the Quebec lakes data of Bird and Kalff (1984). Shortreed and Stockner (1986) also obtained a slope of 0.70 for the log–log regression of bacteria versus Chl *a* for their pooled data compared with the slope of 0.41 ± 0.05 for our study lakes. All the regressions were highly significant ($p < 0.01$), although their data spanned a wider range into more productive lakes. The differences in slopes indicate that the relative increase in bacteria abundance as lake productivity increases is lower in the British Columbia lakes than in the Yukon and Quebec lakes. If we assume that bacterial growth is not limited by conditions unique to the British Columbia lakes, the lower bacterial abundances may reflect higher grazing pressures by bacterivores that have in turn been relieved of some of the predation pressure exerted by the planktivore-cropped mesozooplankton.

The contribution of epilimnetic bacterial carbon biomass to the biomass of the microbial community (phytoplankton plus bacteria) declined from about 24% of the total in the most ultraoligotrophic control lakes to <11% at the top of the range of lake productivities spawned by whole-lake nutrient additions. Inorganic nutrient additions increased phytoplankton biomass relatively more than bacteria biomass. Bacteria thus contribute a substantially higher amount to the combined bacterial and phytoplankton biomass at the lower end of the trophic continuum in oligotrophic British Columbia lakes. Our results agree with the general finding that heterotrophic bacteria, relative to phytoplankton, are more important in (ultra-)oligotrophic than in eutrophic ecosystems (Bird and Kalff 1984; Cole et al. 1988; Simon et al. 1992; Gasol et al. 1995).

Bacterial production and the microbial food web

In spite of the decreased proportional contribution of bacteria to plankton biomass, the relative importance of the microbial community and HNF in particular to the transfer of bacterial production to higher trophic levels appeared to increase with the productivity and abundance of bacteria in the waters over the range of oligotrophic lakes studied. Bacterial production appeared to be closely balanced by grazing by HNF and, to a lesser extent, ciliates and other microzooplankton. Production of bacterial cells was for the most part equivalent to cell losses to grazing HNF in the 1–8 µm size-class.

However, the balance between bacterial growth and grazing losses to protozoans was also sensitive to the abundance of crustacean zooplankton. The microzooplankton appear to be utilizing bacterial production largely through predation on HNF, although the former is a diverse size-class composed of various ciliate and rotifer species with dissimilar feeding mechanisms and trophic relationships. Mesozooplankton such as calanoid and cyclopoid copepods and copepodites were capable of feeding on and suppressing the HNF and microzooplankton assemblage in Shuswap Lake. When copepods reached high concentrations in the experiments, protist populations declined and bacteria had some refuge from grazing. However, recent evidence suggests that mesozooplankton effects on the microbial food web may change rapidly in the same lake depending on complex interactions from both direct feeding and indirect effects such as competition and nutrient recycling (Gasol et al. 1995; Wickham 1998). These types of trophic interactions and cascading effects on the microbial food webs have been described for both marine and freshwater ecosystems (Jürgens et al. 1994; Wickham 1995; Weisse 2000), and they are important processes within the food webs in these oligotrophic British Columbia lakes.

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References

Berman, T. 1990. Microbial food-webs and nutrient cycling in lakes: changing perspectives. *In* Large lakes — ecological structure and function. *Edited by* M.M. Tilzer and S. Serruya. Springer, Berlin. pp. 511–525.

Berninger, U.-G., Finlay, B.J., and Kuuppo-Leinikki, P. 1991. Protozoan control of bacterial abundances in freshwater. Limnol. Oceanogr. **36**: 139–147.

Billen, G., Servais, P., and Becquevort, S. 1990. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia, **207**: 37–42.

Bird, D.F., and Kalff, J. 1984. Empirical relationship between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. **41**: 1015–1023.

Caron, D.A., Lessard, E.J., Voytek, M., and Dennet, M.R. 1993. Use of tritiated thymidine (TdR) to estimate rates of bacterivory:

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implications of label retention and release by bacterivores. Mar. Microb. Food Webs, **7**: 177–196.

Cole, J.J., and Caraco, N.F. 1993. The pelagic foodweb of oligotrophic lakes. *In* Aquatic microbiology. An ecological approach. *Edited by* T.E. Ford. Blackwell Scientific Publications, Cambridge, Mass. pp. 101–111.

Cole, J.J., Findlay, S., and Pace, M.L. 1988. Bacterial production in freshwater and saltwater systems: a cross-system overview. Mar. Ecol. Prog. Ser. **21**: 15–26.

Costella, A.C., Nidle, B., Bocking, R., and Shortreed, K.S. 1982. Limnological results from the 1980 Lake Enrichment Program. Can. MS Rep. Fish. Aquat. Sci. No. 1635.

Costella, A.C., Nidle, B., and Shortreed, K.S. 1983*a*. Limnological results from the 1981 British Columbia Lake Enrichment Program. Can. MS Rep. Fish. Aquat. Sci. No. 1693.

Costella, A.C., Nidle, B., and Shortreed, K.S. 1983*b*. Limnological results from the 1982 British Columbia Lake Enrichment Program. Can. MS Rep. Fish. Aquat. Sci. No. 1706.

Forsberg, C., and Ryding, S.O. 1980. Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. Arch. Hydrobiol. **89**: 189–207.

Fuhrman, J.A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescence microscopy compared to scanning electron microscope. Mar. Ecol. Prog. Ser. **5**: 103–106.

Gasol, J.M., and Vaqué, D. 1993. Lack of coupling between heterotrophic nanoflagellates and bacteria: a general phenomenon across aquatic systems? Limnol. Oceanogr. **38**: 657–665.

Gasol, J.M., Simons, A.M., and Kalff, J. 1995. Patterns in the top-down versus bottom-up regulation of heterotrophic nanoflagellates in temperate lakes. J. Plankton Res. **17**: 1879–1903.

Hyatt, K.D., and Stockner, J.G. 1985. Responses of sockeye salmon (*Oncorhynchus nerka*) to fertilization of British Columbia lakes. Can. J. Fish. Aquat. Sci. **42**: 320–331.

Jürgens, K., Arndt, H., and Rothhaupt, K.O. 1994. Zooplankton-mediated changes of bacterial community structure. Microb. Ecol. **27**: 27–42.

Kirchman, D.L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *In* Handbook of methods in aquatic microbial ecology. *Edited by* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole. Lewis Publishers, Boca Raton, Fla. pp. 509–512.

Kirchman, D.L. and Ducklow, H.W. 1993. Estimating conversion factors for the thymidine and leucine methods for measuring bacterial production. *In* Handbook of methods in aquatic microbial ecology. *Edited by* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole. Lewis Publishers, Boca Raton, Fla. pp. 513–517.

Landry, M.R. 1994. Methods and controls for measuring the grazing impact of planktonic protists. Mar. Microb. Food Webs, **8**(1–2): 37–57.

Lane, P.A. 1978. Role of invertebrate predation in structuring zooplankton communities. Verh. Int. Ver. Limnol. **20**: 480–485.

Lee, S., and Fuhrman, J.A. 1987. Relationships between biovolume and biomass of naturally-derived marine bacterioplankton. Appl. Environ. Microbiol. **52**: 1298–1303.

MacIsaac, E.A., Shortreed, K.S., and Stockner, J.G. 1981. Seasonal distribution of bacterioplankton numbers and activities in eight fertilized or untreated oligotrophic British Columbia lakes. Can. Tech. Rep. Fish. Aquat. Sci. No. 994.

Nidle, B.H., and Shortreed, K.S. 1985. Limnological results from the 1984 British Columbia Lake Enrichment Program. Can. MS Rep. Fish. Aquat. Sci. No. 1834.

Nidle, B.H., Shortreed, K.S., and Masuda, K.V. 1984. Limnological results from the 1983 British Columbia Lake Enrichment Program. Can. MS Rep. Fish. Aquat. Sci. No. 1752.

O'Neill, S.M., and Hyatt, K.D. 1987. An experimental study of competition for food between sockeye salmon (*Oncorhynchus nerka*) and threespine sticklebacks (*Gasterosteus aculeatus*) in a British Columbia coastal lake. *In* Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. *Edited by* H.D. Smith, L. Margolis, and C.C. Wood. Can. Spec. Publ. Fish. Aquat. Sci. No. 96. pp. 143–160.

Porter, K.G., and Feig, Y.S. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25**: 943–948.

Sanders, R.W., Caron, D.A., and Berninger, U.-G. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-system comparison. Mar. Ecol. Prog. Ser. **86**: 1–14.

Shortreed, K.S., and Stockner, J.G. 1986. Trophic status of 19 subarctic lakes in the Yukon Territory. Can. J. Fish. Aquat. Sci. **43**: 797–805.

Simon, M., and Azam, F. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. **51**: 201–213.

Simon, M., Cho, B.C., and Azam, F. 1992. Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. Mar. Ecol. Prog. Ser. **86**: 103–110.

Stephens, K., and Brandstaetter, R. 1983. A laboratory manual: collected methods for the analysis of water. Can. Tech. Rep. Fish. Aquat. Sci. No. 1159.

Stockner, J.G., and MacIsaac, E.A.. 1996. British Columbia lake enrichment programme: two decades of habitat enhancement for sockeye salmon. Regul. Rivers. Res. Manage. **12**: 547–561.

Stockner, J.G., and Shortreed, K.S. 1989. Algal picoplankton production and contribution to food webs in oligotrophic British Columbia lakes. Hydrobiologia, **173**: 151–166.

Thingstad, T.F., Hagstrom, A., and Rassoulzadegan, F. 1997. Accumulation of degradable DOC in surface waters: is it caused by a malfunctioning loop? Limnol. Oceanogr. **42**: 398–404.

Weisse, T. 1989. The microbial loop in the Red Sea: dynamics of pelagic bacteria and heterotrophic nanoflagellates. Mar. Ecol. Prog. Ser. **55**: 241–250.

Weisse, T. 1999. Bacterivory in the northwestern Indian Ocean during intermonsoon – NE monsoon period. Deep-Sea Res. **46**: 795–814.

Weisse, T. 2000. Pelagic microbes — Protozoa and the microbial food web. *In* The lakes handbook. *Edited by* P. O'Sullivan and C.S. Reynolds. Blackwell Scientific Publications, Oxford, U.K. In press.

Weisse, T., Müller, H., Pinto-Coelho, R.M., Schweizer, A., Springmann, D., and Baldringer, G. 1990. Response of the phytoplankton spring bloom in a large prealpine lake. Limnol. Oceanogr. **35**: 781–794.

Wickham, S.A. 1995. Trophic relations between cyclopoid copepods and ciliated protists: complex interactions link the microbial and classic food webs. Limnol. Oceanogr. **40**: 1173–1181.

Wickham, S.A. 1998. The direct and indirect impact of *Daphnia* and *Cyclops* on a freshwater microbial food web. J. Plankton Res. **20**: 739–755.

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Effects of feeding ration on larval swimming speed and responsiveness to predator attacks: implications for cohort survival

John H. Chick and Michael J. Van Den Avyle

Abstract: We conducted laboratory experiments to examine the effects of feeding ration on the routine swimming speed of larval striped bass (*Morone saxatilis*) and their responsiveness to simulated-predator attacks. Striped bass were reared in low (7 prey·L^{-1}), medium (354 prey·L^{-1}), or high (740 prey·L^{-1}) prey treatments from age 4 to 14 days posthatch. Larvae reared in the low-prey treatment had slower routine swimming speeds and shorter reactive distances and were less responsive to simulated-predator attacks. These differences were most pronounced after age 10 and appeared to be an effect of deteriorating larval condition rather than an effect of size. Simulation models were constructed for two potential fish predators, *Alosa aestivalis* and *Pomoxis nigromaculatus*, to examine how variation in growth rate, swimming speed, and responsiveness to predator attacks might influence mortality rate. Our simulations predicted that cohort mortality rate would decrease with increasing larval growth rates, even though faster routine swimming speed and growth rate increased encounter rates with predators. The influence of larval growth rate and responsiveness on mortality rate varied between the two predators, but cohorts experiencing no growth always had the greatest mortality rate.

Résumé : Nous avons réalisé des expériences de laboratoire pour examiner les effets de la ration alimentaire sur la vitesse de nage régulière des larves de bar rayé (*Morone saxatilis*) et leur réactivité à des attaques simulées de prédateurs. Les bars rayés ont été élevés dans des conditions de présence faible (7 proies·L^{-1}), moyenne (354 proies·L^{-1}) et forte (740 proies·L^{-1}) des proies, entre l'âge de 4 et 14 jours après l'éclosion. Les larves élevées dans des conditions de présence faible des proies avaient des vitesses régulières de nage plus basses et des distances de réaction plus courtes et réagissaient moins aux simulations d'attaques de prédateurs. Ces différences étaient plus marquées après l'âge de 10 jours et semblaient être un effet de la détérioration de la condition des larves plutôt qu'un effet de la taille. Les modèles de simulation ont été construits pour deux poissons qui sont des prédateurs potentiels, *Alosa aestivalis* et *Pomoxis nigromaculatus*, en vue d'examiner en quoi la variation du taux de croissance, de la vitesse de nage et de la réactivité pourrait influer sur le taux de mortalité. Nos simulations ont prédit que le taux de mortalité d'une cohorte devait décroître avec l'augmentation du taux de croissance des larves, même si la hausse de la vitesse régulière de nage et du taux de croissance faisait croître le taux de rencontre des prédateurs. L'influence du taux de croissance des larves et de la réactivité sur le taux de mortalité variait selon le prédateur, mais les cohortes dans lesquelles la croissance était nulle étaient toujours celles dont le taux de mortalité était le plus élevé.

[Traduit par la Rédaction]

Introduction

Cohorts of larval striped bass (*Morone saxatilis*) encounter spatial and temporal variation in prey abundance and composition that can affect their feeding success and growth (Rulifson and Manooch 1990; McGovern and Olney 1996). Prey availability can affect cohort mortality rate directly through starvation and indirectly through the effects of larval growth rate and condition on larval vulnerability to predators and disease. Research on the Lake Marion, South Carolina, U.S.A., striped bass population suggests that spatial variation of zooplankton may have substantial effects on the growth rates of cohorts transported to different areas along the riverine to lentic gradient in the upper portion of the system (Bulak et al. 1997; Chick and Van Den Avyle 1999*a*, 1999*b*). For this study, we examined how differences in feeding ration might affect the vulnerability of striped bass larvae to predators.

The exact role that size and growth play in the vulnerability of fish larvae to predators is a continuing source of

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Table 1. Summary of growth rate (mm·day^{-1}), mortality rate (%·day^{-1}), and ratio of dry weight (DW, g) to TL (mm) for striped bass larvae reared under high, medium, and low prey abundances from ages 4 to 14 days posthatch (Chick and Van Den Avyle 1999*b*).

Treatment	Growth	Mortality	DW/TL, age 6	DW/TL, age 14
Experiment 1 (April 7–17, 1996)				
High	0.03	5	0.017	0.015
Medium	0.02	5	0.020	0.013
Low	–0.01	7	0.018	0.011
Experiment 2 (April 4 – May 1, 1996)				
High	0.17	5	0.018	0.032
Medium	0.15	4	0.017	0.030
Low	–0.01	11	0.024	0.012
Experiment 3 (May 14–24, 1996)				
High	0.13	8	0.023	0.036
Medium	0.09	4	0.019	0.027
Low	–0.02	21	0.020	0.012

debate (Bailey and Houde 1989; Leggett and Deblois 1994). Vulnerability to predators is believed to decrease with growth rate because the number of potential predators declines and escapement abilities improve as larvae grow (Miller et al. 1988; Bailey and Houde 1989). Faster growing cohorts are presumed to have better survival because less time is spent in stages with high vulnerability to predation. Rapid growth and increased body size, however, have other effects that may increase larval vulnerability to predators. As body size increases, routine swimming speed and visibility to predators increase, which could lead to more frequent encounters with predators (Bailey and Batty 1983; Bailey and Houde 1989; Cowan et al. 1996; Letcher et al. 1996). Furthermore, larger size may be detrimental if predators preferentially attack larger larvae (Litvak and Leggett 1992).

Compared with the effects of larval size and growth, relatively little research has addressed how non-size-related nutritional factors affect larval vulnerability to predators. Prey availability may influence larval–predator interactions through non-size-related effects on the condition, activity level, and physiological development of larvae. Our objective for this study was to examine the effect of feeding ration on larval striped bass interactions with simulated predators. We conducted laboratory experiments assessing routine swimming speed, which affects encounter rates with predators, and responsiveness to predator attacks. To better understand the potential impacts of both size- and non-size related effects of feeding ration on larval vulnerability to predators, we developed simulation models to predict mortality rates from two potential fish predators.

Materials and methods

Experimental protocol

Methods used to rear the larvae have been described previously and will only be briefly summarized here (Chick and Van Den Avyle 1999*b*). Three-day-old striped bass larvae from the spawning of three female and nine male striped bass were obtained from the J.D. Bayless Hatchery, South Carolina. We reared larvae in blue plastic containers filled with 15 L of water from ages 4 to 14 days posthatch. Each container was stocked with 50 age-3 larvae (3.33 larvae·L^{-1}). Starting at age 5, zooplankton was added to the containers in three treatments: low (6 micro- and 1 macro-zooplankton·L^{-1}), medium (350 micro- and 4 macro-zooplankton·L^{-1}), and high (700 micro- and 40 macro-zooplankton·L^{-1}). These treatments were selected to represent a range of zooplankton conditions similar to those found in upper Lake Marion, South Carolina (Chick and Van Den Avyle 1999*a*, 1999*b*).

Growth and mortality estimates were derived from larvae harvested from three containers for each treatment at 2-day intervals beginning at age 6 and ending at age 14. Larvae were unable to grow in the low treatment but grew at rates ranging from 0.02 to 0.17 mm·day^{-1} in the medium and high treatments. Additionally, the ratio of dry weight to length consistently decreased for larvae in the low treatment, suggesting that their condition was deteriorating (Table 1) (Chick and Van Den Avyle 1999*b*).

At 2-day intervals beginning at age 6, we randomly subsampled six larvae from each feeding treatment and measured their routine swimming speed and responsiveness to attacks by simulated predators. Observations of individual larvae (270 in total) were made in a 15-cm petri dish using an 8-mm video camera mounted 1.5 m above the dish. Overhead lighting from a 50-W incandescent spotlight provided a light intensity of 7 μeinsteins·m^{-2}·s^{-1} in the petri dish. Disturbance from other light sources was minimized by enclosing the area with a black plastic curtain. We filled the petri dish with 150 mL of water (22–25°C) and zooplankton from each larva's rearing container. These temperature and lighting conditions were typical of those that we have observed in Lake Marion during the spring and were similar to the conditions in the rearing containers (Chick and Van Den Avyle 1999*a*, 1999*b*).

To facilitate measurement of routine swimming speed and responsiveness, the petri dish was placed on blue paper marked with a grid of 6-mm squares. We transferred larvae to the petri dish with a plastic ladle and allowed them to acclimate for 5 min. This acclimation period was chosen because in pilot studies, most larvae resumed feeding within 5 min after transfer. After acclimation, activity was recorded for 2 min and we quantified routine swimming speed (millimetres per second) from the total number of grids crossed. We used a two-way ANOVA to examine whether the routine swimming speed varied among feeding treatments, larval age, and the interactive effects of age and feeding treatment. Routine swimming speed was log transformed to comply with assumptions of normality and homoscedasticity. We repeated the experiment three times during April and May of 1996 and conducted separate analyses for each repetition of the experiment because several factors, including brood, temperature, and zooplankton composition, varied among experiments (Chick and Van Den Avyle 1999*b*).

After filming swimming behavior for 2 min, we simulated predator attacks by approaching larvae with a model predator and recording their responses (Blaxter and Fuiman 1989; Higgs and Fuiman 1996). The model predator consisted of the head of a fishing lure, resembling a 30-mm clupeid, mounted on the tip of a pasteur pipet. We manually advanced the model within the larva's forward field of vision at a speed of 7–10 cm·s^{-1}. If a larva did not respond to the initial approach or contact with the model predator, we attacked it up to two more times. Higgs and Fuiman (1996) reported that the variation of approach speed associated with manually advancing a model predator did not significantly affect responsiveness, and larvae in our pilot studies did not respond to movements by the person manipulating the model predator.

We quantified three aspects of the responsiveness of larvae to predator attacks: (*i*) the number of larvae responding to the first attack, (*ii*) the number failing to respond to any attack, and (*iii*) reactive distance, which was measured as the distance between the model and the larva when the larva initiated a burst swimming response. Reactive distance was quantified for the first attack that a larva responded to. Computer images were generated from video

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recordings, and we measured reactive distance to the nearest 0.1 mm using image analysis software. We used ANOVA to examine whether the number of larvae responding to the first attack and the number failing to respond to an attack varied among the three feeding treatments or with age. No data transformation appeared necessary based on examination of residual plots. We used a two-way ANOVA to examine whether the reactive distance varied among feeding treatments, larval age, and the interactive effects of age and feeding treatment. Reactive distance was square root transformed to comply with assumptions of normality and homoscedasticity. We used regression analysis to examine relationships of swimming speed and reactive distance with total length and Fuiman's (1994) ontogenetic index.

Several laboratory studies, using petri dishes or small experimental arenas, have successfully demonstrated variation in routine swimming speed and responsiveness to predator attack with larval size or ontogeny (Rosenthal and Hempel 1973; Fuiman and Webb 1988; Blaxter and Fuiman 1989; Higgs and Fuiman 1996). Nevertheless, routine swimming speed of larvae in these settings likely differs from that in the field due to water currents and turbulence, so laboratory measures should be interpreted as rough approximations. We feel that our experimental design was appropriate for detecting treatment effects and that the size range of the larvae in our experiments, 4.90–7.23 mm standard length, was small enough to avoid size-related biases associated with the container.

Simulation model

We developed a simulation model to better understand how feeding ration, through its influence on growth, swimming speed, and responsiveness to predator attacks, might affect cohort mortality. Specifically, we examined the sensitivity of the predicted overall mortality rate, encounter rate, and capture probability to the variation in routine swimming speed, growth rate, and responsiveness observed in our experiments.

Our model was based on a simple mathematical equation that predicts changes in the size of a larval cohort though time:

$$N_t = (1 - p - \mathrm{m})^t N_0$$

where N_t is the number of larvae at time t, N_0 is the initial number of larvae, p is the probability of predation mortality, and m is the probability of mortality from nonpredator factors. By definition, $p + m$ cannot be greater than 1. The p was a function of encounter rates between larvae and predators, the probability of predators attacking encountered larvae, and the probability of predators capturing larvae that they attack. Thus, p can be determined from

$$p = EaC$$

where E is the encounter rate with predators per day, a is the probability of attack, and C is the capture probability. By definition, $0 \leq p \leq 1$.

Model formulation

For each day of the simulation, the model determined the total number of larvae in a cohort that died. We assumed that all larvae grew at the same rate (G_L, millimetres per day) and all predators were of identical size (L_P, millimetres). To calculate the rate of encounter between larvae and predators, we used the formula first described by Gerritsen and Strickler (1977) and later modified by Bailey and Batty (1983) to account for larval size. This approach models encounter rate as a function of the volume "searched" by predators and larvae, which is calculated from their encounter radii and routine swimming speeds. The number of predators that a larva encountered in a day, E, was calculated as

$$E = \pi(R_L + R_P)^2\left(\frac{S_L^2 + 3S_P^2}{3S_P^2}\right)D_P$$

where R_L was the encounter radius of the larva (millimetres), R_P was the encounter radius of the predator (millimetres), D_P was the density of predators (number per cubic millimetre), S_L was larval swimming speed (millimetres per day), and S_P was the swimming speed of the predator (millimetres per day). This equation assumes that (*i*) predators and larvae are randomly distributed, (*ii*) predators and larvae swim in random directions within the modeled volume, (*iii*) the volume searched by a predator is cylindrical with radius R_P and length S_P, and (*iv*) the encounter volume of a larva is a sphere with radius R_L (Gerritsen and Strickler 1977; Bailey and Batty 1983; Cowan et al. 1996).

For each encounter between a larva and a predator, we assumed a constant probability, a, that the predator would attack. The probability that a larva would be captured was calculated from Miller et al. (1988)'s empirically derived capture success function for predators of fish larvae:

$$C = \left[100 - \left(\frac{L_P/L_L + 3.37}{44.76}\right)^{-2.28}\right] \times 10^{-2}$$

where L_P was the length of the predator (millimetres) and L_L was the length of the larva (millimetres). Larvae also had a finite chance of dying, m, from nonpredator sources.

Parameter estimation

Because we assigned larval swimming speed and growth rates based on our laboratory experiments, our model is specific to the temperature, light intensity, and water quality conditions described above. To simulate the effects of reduced responsiveness, we increased C, which was calculated based solely on larval and predator size, incrementally up to 0.99 for larvae experiencing no growth. We assigned values for S_L based on the results of our routine swimming experiments and examined how this affected the predicted encounter rates and cohort mortality. Cohorts with zero growth were assigned an initial routine swimming speed of 1.2 $L_L \cdot s^{-1}$ and a speed of 0.9 $L_L \cdot s^{-1}$ after age 10. Routine swimming speed remained at 1.2 $L_L \cdot s^{-1}$ for all ages for cohorts with positive growth rates.

The model began with larval total length (TL) = 4.5 mm at age 5 and an initial cohort size of 2000, and the modeled volume was 200 m^3. We ran simulations using four cohort growth rates, G_L = 0, 0.14, 0.25, and 0.46 mm·day^{-1}, based on our growth experiments and other reported striped bass growth rates (Secor et al. 1995). We ran simulations where mortality from nonpredator factors, m, was set at 0.05·day^{-1} (5%·day^{-1}) for all growth rates and simulations where m was increased to 0.13·day^{-1} (12%·day^{-1}) for cohorts with G_L = 0 to mimic our experimental results (Table 1) (Chick and Van Den Avyle 1999*b*). We calculated the daily mortality coefficient, Z, from the equation $Z = (\ln N_0 - \ln N_f)/t$ where N_0 is the initial number of larvae and N_f is the mean number of larvae at age 29.

We ran separate simulations with two types of vertebrate predators known to occupy nursery habitats in upper Lake Marion. The first predator was age-0 blueback herring (*Alosa aestivalis*) 30 mm TL (Table 2). Blueback herring are pelagic planktivores that consume fish larvae (Grabe 1996). We assumed that blueback herring would swim at 1.5 lengths·s^{-1}, a rate typical for this species and other clupeids of similar size (Rosenthal and Hempel 1973; Janssen 1982). Grabe (1996) reported that blueback herring either feed little at night or switch to a pump-filter feeding mode. Therefore, we simulated this predator foraging at a constant rate for 12 h each day ($S_{P1} = 1.5L_{P1} \times 43\,200$) (Table 2). The density of blueback herring within the modeled volume was 0.875·m^{-3} ($D_{P1} = 8.75 \times 10^{-10}$·$mm^{-3}$), a realistic density for upper Lake Marion during the spring (Bulak and Crane 1991).

The second predator modeled was age-1 black crappie (*Pomoxis nigromaculatus*). Black crappie are generally planktivorous until

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Table 2. Parameter calibrations and initial values of variables for simulation models of larval striped bass cohort mortality.

Description	Symbol	*Alosa aestivalis*	*Pomoxis nigromaculatus*
Initial number of larvae	N_L	2000	2000
Initial length of larvae (mm)	L_L	4.5	4.5
Predator length (mm)	L_P	30	78
Predator density (number·mm^{-3})	D_P	8.75×10^{-10}	7.5×10^{-11}
Swimming speed of larvae	S_L	$1.2L_L \times 43\,200$, $0.9L_L \times 43\,200$	$1.2L_L \times 43\,200$, $0.9L_L \times 43\,200$
Swimming speed of predators	S_P	$1.5L_P \times 43\,200$	$0.5L_P \times 43\,200$
Attack probability	a	0.1	0.1
Probability of nonpredator mortality	m	0.05, 0.13	0.05, 0.13
Larval growth rate (mm·day^{-1})	G_L	0, 0.14, 0.25, 0.46	0, 0.14, 0.25, 0.46

Note: Separate models were generated to simulate predation from two potential fish predators, *A. aestivalis* and *P. nigromaculatus*, known to cooccur with larval striped bass in Lake Marion.

they reach about 150 mm TL but can begin feeding on larval fish by the time they reach 15 mm TL (Hanson and Qadri 1984). Black crappie utilize a saltatory foraging behavior, and we assumed that they would swim at a rate of 0.5 length·s^{-1}, a typical rate for saltatory planktivores (Janssen 1982; O'brien et al. 1989). Zooplanktivorous black crappie apparently do not feed at night (Hanson and Qadri 1984); therefore, we assumed a constant foraging rate for 12 h each day ($S_{P2} = 0.5L_{P2} \times 43\,200$) (Table 2). We chose 78 mm TL as the length for this predator and set the density in the modeled volume at 0.075·m^{-3} ($D_{P2} = 7.5 \times 10^{-11}$·mm^{-3}), a realistic size and density for black crappie in upper Lake Marion during the spring (Bulak and Crane 1991).

We set the encounter radius, R_P, of both predators as $0.8L_P$ and the encounter radius of a larva as $R_L = 2L_L/\pi^2$ (Bailey and Batty 1983; Cowan et al. 1996). We assumed that alternative prey (zooplankton) was available and would reduce the number of larvae consumed by predators, as has been found in several studies (Kean-Howie et al. 1988; Gotceitas and Brown 1993). For example, Kean-Howie et al. (1988) found that when alternative zooplankton prey was two orders of magnitude more abundant than larval white perch (*Morone americana*), bluegill (*Lepomis macrochirus*) consumed only about 7% as many larvae as when the abundances of larvae and zooplankton were equal. Rather than affecting the encounter rate or capture probability, we assumed that the presence of alternative prey would alter the probability of a larva being attacked (i.e., the predator may be encountering, attacking, or consuming an alternative prey item when the larva encounters it). Because zooplankton are often 10^2–10^4 times more abundant than larval striped bass in upper Lake Marion (Bulak and Crane 1991; Chick and Van Den Avyle 1999*a*), we assumed that the attack probability of both predators would be relatively low and set a = 0.1 (Table 2). We assumed that predators would not become satiated from foraging on larvae and zooplankton.

Results

Experiments

Routine swimming speed and all measures of responsiveness to predators were significantly affected by feeding treatment. Larvae in the low treatment generally had slower swimming speeds than those in the high and medium treatments. Average swimming speed ranged from 4.8 to 5.7 mm·s^{-1} in the low treatment, from 4.7 to 7.3 mm·s^{-1} in the medium treatment, and from 6.1 to 8.9 mm·s^{-1} in the high treatment. Differences among treatments were most apparent after age 10 and were significant in experiments 1 and 2 (Table 3; Fig. 1). Differences among ages were significant in experiments 1 and 3, and age interacted significantly with treatment in experiment 3. There was little evidence, however, of any overall increase or decrease of routine swimming speed with age (Fig. 1). Swimming speed increased significantly with length (P = 0.0002) and the ontogenetic index (P = 0.0001), but these relationship were highly variable (R^2 = 0.05, 0.12).

Well-fed larvae had greater reactive distances than poorly fed larvae, and differences among feeding treatments were significant in all three experiments. The mean reactive distance did not vary significantly between the high and medium treatments (Tukey's, $P > 0.05$), but these larvae had significantly greater reactive distances than those in the low treatment (Tukey's, $P \leq 0.05$). Once again, differences among treatments were most apparent after age 10 when mean reactive distance was ≤2 mm in the low treatment versus 5–15 mm in the high and medium treatments (Fig. 1). As with swimming speed, there was little evidence of any overall trend in reactive distance with age, and the only significant differences among ages occurred in experiment 1 (Fig. 1; Table 3). Age interacted significantly with feeding treatment in experiment 2, probably as a result of the sharp decrease of reactive distance in the low treatment after age 10. Reactive distance increased significantly with length (P = 0.004) and the ontogenetic index (P = 0.0001), but these relationships were highly variable (R^2 = 0.03, 0.11).

Larvae in the low-feeding treatment were less responsive to attacks by the model predator. Both the number of larvae responding to the first attack and the number of larvae failing to respond to any attack varied significantly among the feeding treatments in experiments 2 and 3 (Table 3; Fig. 2). In these experiments, the number of larvae responding to the first attack was significantly less (Tukey's, $P \leq 0.05$) and the number of larvae never responding was significantly greater (Tukey's, $P \leq 0.05$) in the low than in the high and medium treatments. Differences between the medium and high treatments were not significant (Tukey's, $P > 0.05$). As with routine swimming speed, differences among the feeding treatments were most apparent after age 10 when fewer than half of the larvae in the low treatment responded to the first attack and as many as half never responded to any attack (Fig. 2). In contrast, after age 10, a majority of larvae in the high and medium treatments responded to the first attack, and no larva from these treatments failed to respond to any attack.

Simulations

In our simulations, increasing growth rates led to reduced

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Table 3. Results from ANOVA testing the effects of larval age and feeding treatment on the number of striped bass larvae responding to the first predator attack (first attack), the number of larvae never responding to an attack (no response), routine swimming speed, and the reactive distance of larvae.

		P			*P*	
Source	df	First attack	No response	df	Swimming speed	Reactive distance
Experiment 1						
Model	6	0.3000	0.0080	14	0.0001	0.0001
Age	4	0.3220	0.0060	4	0.0001	0.0001
Treatment	2	0.2534	0.0649	2	0.0060	0.0093
Age × treatment				8	0.0964	0.0674
Error	8			75		
R^2		0.52	0.84		0.42	0.46
Experiment 2						
Model	6	0.0196	0.0435	14	0.0005	0.0003
Age	4	0.6471	0.4222	4	0.2656	0.1389
Treatment	2	0.0091	0.0085	2	0.0001	0.0003
Age × treatment				8	0.1591	0.0085
Error	8			75		
R^2		0.58	0.74		0.38	0.39
Experiment 3						
Model	6	0.0146	0.0499	14	0.0010	0.0076
Age	4	0.7740	0.5512	4	0.0053	0.4105
Treatment	2	0.0115	0.0086	2	0.1400	0.0005
Age × treatment				8	0.0091	0.1364
Error	8			75		
R^2		0.69	0.73		0.36	0.31

cohort mortality rates despite the fact that encounter rates increased with growth rate. Mortality was greater for cohorts experiencing zero growth than for all cohorts with positive growth for both predators (Figs. 3*a* and 3*b*). Larval growth rate had substantial effects on cohort mortality with *Alosa* as the predator but had less of an influence in *Pomoxis* simulations. Increasing nonpredator mortality for cohorts experiencing zero growth further accentuated the differences in cohort mortality (Figs. 3*c* and 3*d*). The predicted mortality rate, Z, with *Alosa* ranged from 0.134·day^{-1} at G_L = 0.46 mm·day^{-1} to 0.330·day^{-1} at G_L = 0 mm·day^{-1} (Fig. 3*c*). With *Pomoxis* as the predator, Z ranged from 0.179·day^{-1} at G_L = 0.46 mm·day^{-1} to 0.289·day^{-1} at G_L = 0 mm·day^{-1} (Fig. 3*d*).

Our model suggests that the differences in routine swimming speed observed in our experiment would have little effect on mortality from the two predators modeled. Because the swimming speed of both predators was much greater than that of the larvae, variation in larval swimming speed and growth had little effect on encounter rate (Fig. 4*a*). For example, a 4.5 mm TL larva with a routine swimming speed of 1.2 L_L·s^{-1} encountered 3.33 *Alosa* or 1.48 *Pomoxis*·day^{-1}. With swimming speed reduced to 0.9 L_L·s^{-1}, the encounter rate was similar: 3.32 *Alosa* and 1.47 *Pomoxis*·day^{-1}. Encounter rates with both predators did increase as larvae grew, but differences in encounter rate never exceeded 0.84 predators·day^{-1} for the range of growth rates modeled (Fig. 4*a*).

The potential for the variation in larval responsiveness observed in our experiments to affect mortality rate varied between the two predators. The capture probability, based on size only, of cohorts with zero growth was constant across all ages at 0.69 for *Alosa* and 0.94 for *Pomoxis* (Fig. 4*b*). This suggests that reduced responsiveness could potentially increase the capture probability by 0.3 (i.e., 0.99 – 0.69) for *Alosa* but only by 0.05 (i.e., 0.99 – 0.94) for *Pomoxis*. For our simulations with *Alosa*, our model predicts that cohorts with zero growth and C = 0.69 would go extinct after age 23 (Fig. 3*c*). Increasing C changed the cohort extinction date to age 22 with C = 0.79, age 21 with C = 0.89, and age 20 with C = 0.99. With *Pomoxis* as the predator, cohort extinction occurred after age 27 for the zero growth cohort (Fig. 3*d*) and occurred after age 26 when C was increased to 0.99. In comparison, differences in growth rate had pronounced effects on capture probability, although these effects were greater for *Alosa* than for *Pomoxis* (Fig. 4*b*).

Discussion

Differences among the feeding treatments in swimming speed and three behavioral indices of responsiveness to predator attack likely were influenced by deteriorating condition of larvae in the low-feeding treatment. We found little evidence that these differences were an effect of larval size or ontogeny. For example, swimming speed of larvae in the low treatment was usually slower at age 14 than at age 6, even though mean length at these two ages was similar (TL_6 = 5.51 mm, TL_{14} = 5.34 mm). Similarly, larvae in the low treatment were also less responsive to simulated predator attacks at age 14 than at age 6. Although we did not directly assess condition, the ratio of dry weight to length consistently decreased with age for larvae in the low treatment, suggesting that their condition was deteriorating (Table 1).

How might reductions in growth, routine swimming speeds, and responsiveness to predator attacks affect larval

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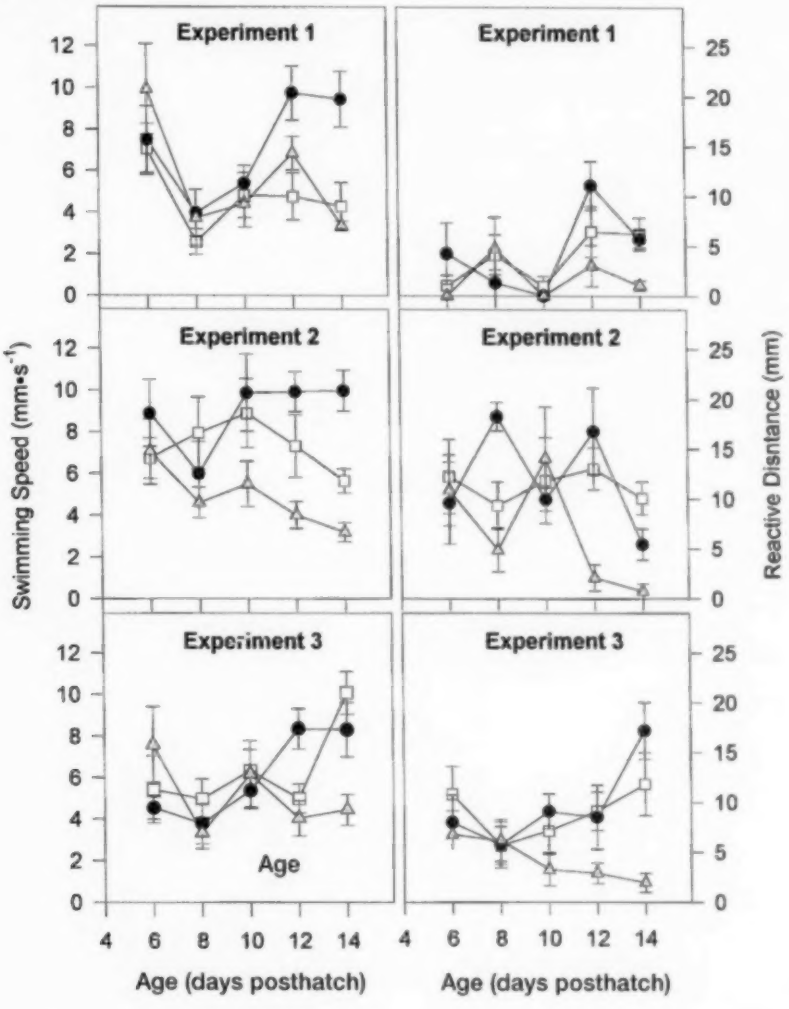
Fig. 1. Routine swimming speed and reactive distance of striped bass larvae reared under three feeding treatments: high (circles), medium (squares), and low (triangles). Swimming speed and reactive distance were determined from six larvae from each treatment for each age. Error bars are ±1 SE.



mortality from predators? In our simulations, routine swimming speed of larvae had only a modest influence on encounter rate with either predator. In contrast, growth rate substantially influenced capture probability, but the overall effects of larval growth likely will vary depending on the species and size of predator. For example, growth rate had a greater influence on mortality from *Alosa* than from *Pomoxis*. In order for cohorts growing at 0.46 mm·day^{-1} to experience the same predation mortality as cohorts with zero growth, encounter rate or attack probability would need to be increased 158% for *Alosa* but only 18% for *Pomoxis*. Similarly, differences in responsiveness and reactive distances had a greater potential to affect mortality from *Alosa* than from *Pomoxis*. Overall, our simulation results suggest that faster growing cohorts will have lower mortality rates than slower growing cohorts. For the predators that we modeled, the benefits of faster growth, namely reduced capture probability, outweighed the detrimental effect of increased encounter rate with predators.

On the surface, these results appear to concur with the premiss that larger and faster growing larvae are less vulnerable to predation, which has been labeled somewhat pejoratively as "the bigger-is-better hypothesis." (Litvak and Leggett 1992; Leggett and Deblois 1994). This hypothesis has received much criticism because several studies have reported that vulnerability to predators was unrelated to, or actually increased with, larval size (Fuiman and Gamble 1989; Cowan and Houde 1992; Litvak and Leggett 1992; Paradis et al. 1996). It appears that the effects of larval growth rate and size on vulnerability to predators are scale dependent. Several studies indicate that cohort mortality decreases with growth rate, even though larger and faster growing individuals within a cohort may have greater mortality rates than smaller and slower growing individuals (Bertram 1996;

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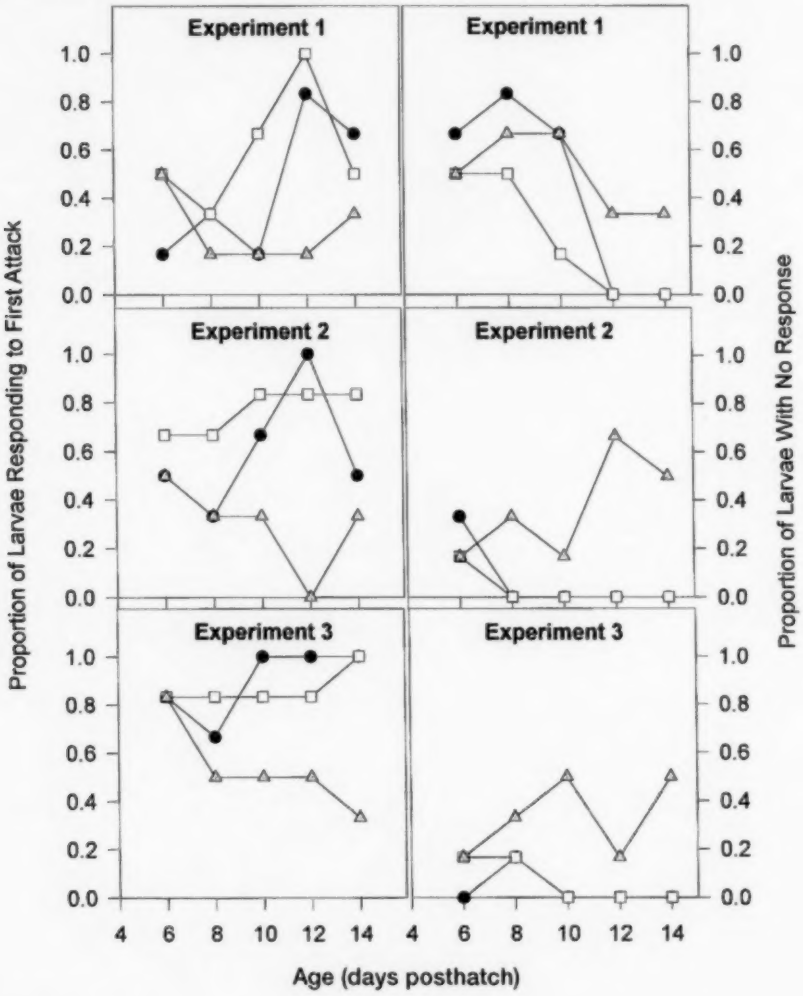
Fig. 2. Variation in the proportion of striped bass larvae responding to the first attack of the model predator, and the proportion never responding to any attack, with age, feeding treatment (high (circles), medium (squares), and low (triangles)), and experiment. Each proportion was calculated from six larvae from each treatment for each age.



Cowan et al. 1996). In addition to size and growth rate, larval condition likely also varies among individuals within a cohort. How variation in condition affects individual and cohort mortality rates is poorly understood, and our study suggests that effects may vary among predator species.

The effect of larval condition on vulnerability to predators is poorly understood because ecologists have attempted to establish a conceptual and empirical framework for larval–predator interactions based on body size (Miller et al. 1988; Paradis et al. 1996). Relatively few laboratory, field, or simulation studies have focused on how condition affects vulnerability to predators (but see Elliott and Leggett 1998). Reasons for the emphasis on body size include the importance of gape limitation for aquatic vertebrate predators, the long history of research on larval growth rate and recruitment, and strong empirical evidence that predator capture success depends on the ratio of larval size to predator size (reviewed by Miller et al. 1988; Leggett and Deblois 1994). Nevertheless, our understanding of the interactions between larval foraging, growth, and predation mortality might be improved by further research on condition and other nonsize-related nutritional factors.

Few studies have examined routine swimming speed as a function of larval condition. Laurence (1972) found reductions in sustained swimming speed, activity level, and routine swimming speed of starved largemouth bass (*Micropterus salmoides*) larvae. Changes in routine swimming speed became apparent after 4 days of starvation, which is similar to the pattern that we observed in the low treatment. We are unaware of any other studies examining routine swimming speed of fish larvae as a function of condition. Several studies have reported increases in routine swimming speed with length (Rosenthal and Hempel 1973; Fuiman and Webb 1988). The weak relationship between routine swimming

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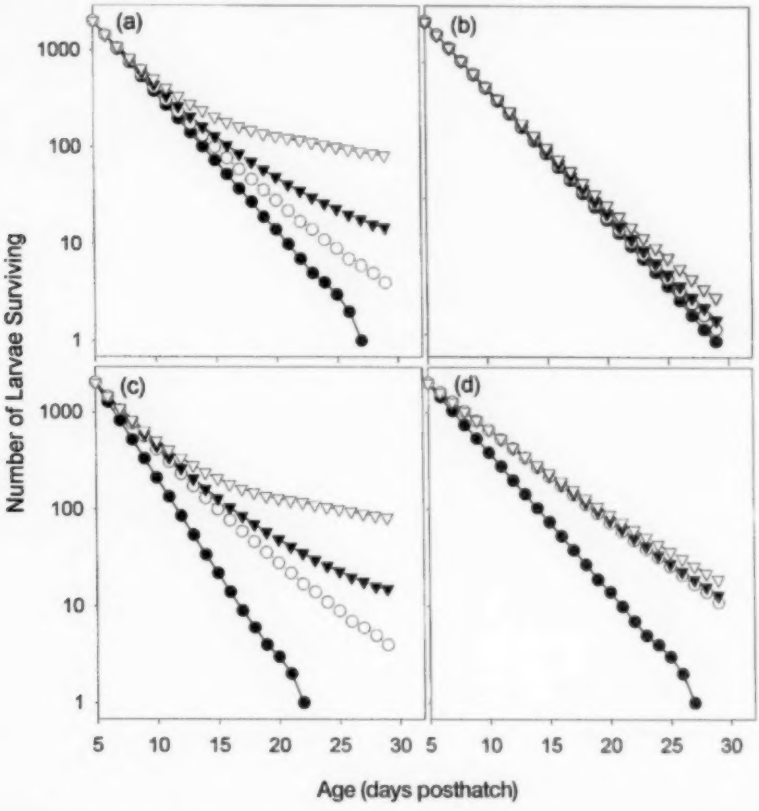
Fig. 3. Predicted number of striped bass larvae surviving from age 5 to age 29 days posthatch growing at rates of 0 (solid circles), 0.14 (open circles), 0.25 (solid triangles), and 0.46 mm·day^{-1} (open triangles) with (*a*) *A. aestivalis* as predator and other mortality (*m*) equal for all growth rates, (*b*) *P. nigromaculatus* as predator and *m* equal for all growth rates, (*c*) *A. aestivalis* as predator and *m* greater for the 0 mm·day^{-1} cohort (see Table 1), and (*d*) *P. nigromaculatus* as predator and *m* greater for the 0 mm·day^{-1} cohort.



speed and length or ontogeny in our study may be the result of our use of relatively narrow ranges of age and size.

As with routine swimming speed, most of the research on larval responsiveness to predator attacks has investigated changes with size and age rather than with condition (Blaxter and Fuiman 1989; Fuiman 1993, 1994; Higgs and Fuiman 1996). Neilson et al. (1986) found that the ability of Atlantic cod (*Gadus morhua*) larvae to avoid a model predator was positively related to feeding ration but was not related to two measures of condition. Yin and Blaxter (1987) found decreased responsiveness and escape speed for starved herring, cod, and flounder. The effects of starvation were not evident until larvae had starved for several days, which is similar to the patterns that we observed in the low treatment.

The relationship between reactive distance and size appears to be species specific. Higgs and Fuiman (1996) found that reactive distance increased with size for Atlantic menhaden (*Brevoortia tyrannus*), whereas Fuiman (1993, 1994) found that reactive distance decreased with size for Atlantic herring (*Clupea harengus*). The effectiveness of *Clupea* at avoiding capture, however, increased with size. We observed concurrent decreases in routine swimming speed, responsiveness to attacks, and dry weight to length ratio for larvae in the low-feeding treatment, suggestive of deteriorating condition. It is doubtful that response effectiveness increased as the reactive distance of these larvae decreased. Optimal reactive distance appears to fall within a narrow range, and reactive distances that are either too short or too long may increase vulnerability to predators (Blaxter and Fuiman 1989; Fuiman 1993).

In our modeling efforts, we assumed that attack probability would not vary with larval size. This assumption is not in accordance with studies suggesting that predators preferentially attack larger sized larvae (Litvak and Leggett 1992; Bertram 1996). The predators that we modeled, however, are primarily zooplanktivores rather than specialist feeders on larval fish. For these predators, any larval fish would likely represent a large prey item. Nevertheless, altering the assumption of equal attack probabilities would certainly influence the results of our simulation model.

Modeling predation mortality as the result of encounter rate and the probabilities of attack and capture has become a common approach of researchers studying larval fishes (Bailey and Batty 1983; Cowan and Houde 1992; Rice et al. 1993; Cowan et al. 1996; Letcher et al. 1996). Usually, empirical relationships are not available for all three components of predation mortality, and assumptions are made about one or more of these parameters (Table 4). Our ability

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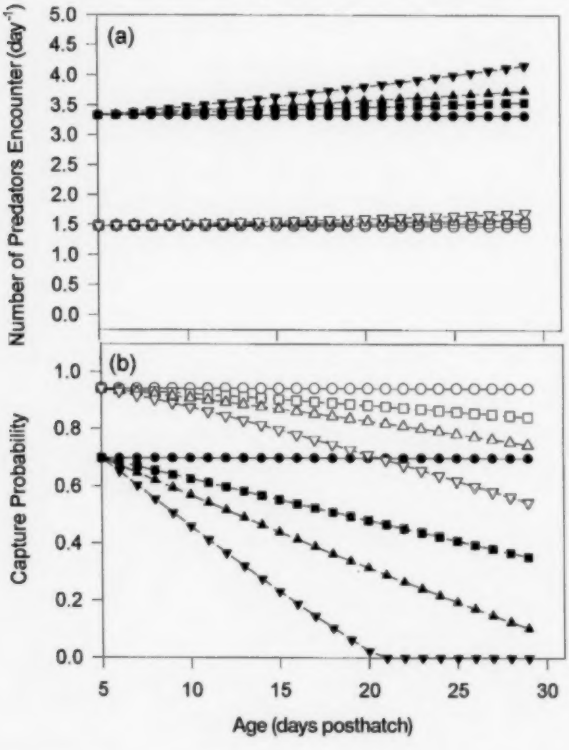
Table 4. Approaches used by simulation studies examining the vulnerability of larval fish to predators.

Study	Approach used			Effects examined
	Encounter rate	a	C	
Bailey and Batty 1983	G&S	Equal to encounter rate	Observed	Model development
Fuiman and Gamble 1989	G&S	Assumed	Observed	Larval density and size
Cowan and Houde 1992	G&S	$a = 1$ for gelatinous predators; realized capture probability ($a + C$) estimated from experiments and encounter model		Larval size, multiple predators
Rice et al. 1993	Assumed	Published model	Published empirical model	Larval size
Cowan et al. 1996	G&S	Realized capture probability ($a + C$) estimated from experiments and encounter model		Larval size, multiple predators
Letcher et al. 1996	G&S	Assumed	Published empirical model	Larval size
This study	G&S	Assumed	Published empirical model	Larval size and condition

Note: The probability of a predator attacking an encountered larva is denoted as a. The probability that an attacked larva will be successfully captured a larva is denoted as C. G&S indicates usage of the Gerritsen and Strickler (1977) encounter rate model.

Fig. 4. (*a*) Predicted number of predators encountered per day by striped bass larvae growing at 0 (circles), 0.14 (squares), 0.25 (triangles), and 0.46 mm·day^{-1} (triangles) and (*b*) probability of a predator capturing a striped bass larvae growing at 0, 0.14, 0.25, and 0.46 mm·day^{-1}. Solid symbols correspond to simulations with *A. aestivalis* as the predator, and open symbols correspond to simulations with *P. nigromaculatus* as the predator.



to model encounter rate and attack probability might be improved by combined laboratory and mesocosm experiments. Capture probability and predator encounter radius can be quantified in laboratory settings (Miller et al. 1988; Fuiman and Gamble 1989). Once the encounter radius of a predator is known, videography could be used to estimate attack probability based on the proportion of larvae that are attacked after entering the defined encounter radius of the predator. These efforts should be conducted in the presence of alternative prey to improve realism. With encounter radius and attack and capture probabilities defined empirically, our ability to model encounter rate could be tested and refined by comparing the results of mesocosm experiments with predictions from simulation models.

In our study, larval condition appeared to be more important than size to the behavioral indices that we measured, and our simulations predicted that faster growing cohorts should have lower mortality rates than slower growing cohorts. More information is needed on how larval condition affects encounter rates, attack probabilities, and mortality from multiple species of predators. The focus on how size affects vulnerability is useful, but it is not the only effect of prey availability that can influence larval vulnerability to predators. Furthermore, the influence of larval size and condition on vulnerability likely will vary with the species and sizes of predators present.

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References

Bailey, K.M., and Batty, R.S. 1983. A laboratory study of preda-

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tion by *Aurelia aurita* on larval herring (*Clupea harengus*): experimental observations compared with model predictions. Mar. Biol. **72**: 295–301.

Bailey, K.M., and Houde, E.D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Bertram, D.F. 1996. Size-dependent predation risk in larval fishes: mechanistic inferences and levels of analysis. Fish. Bull. U.S. **94**: 371–373.

Blaxter, J.H.S., and Fuiman, L.A. 1989. Function of free neuromasts of marine teleost larvae. *In* The mechanosensory lateral line: neurobiology and evolution. *Edited by* S. Coombs, P. Gorner, and H. Munz. Springer-Verlag, New York. pp. 481–499.

Bulak, J.S., and Crane, J.S. 1991. An assessment of the ichthyoplankton of Santee River and the headwaters of Lake Marion, South Carolina, with emphasis on striped bass. S.C. Wildl. Mar. Resour. Dep. Spec. Completion Rep. WC-5.

Bulak, J.S., Crane, J.S., Secor, D.H., and Dean, J.M. 1997. Recruitment dynamics of striped bass in the Santee–Cooper system, South Carolina. Trans. Am. Fish. Soc. **126**: 133–143.

Chick, J.H., and Van Den Avyle, M.J. 1999*a*. Zooplankton variability and larval striped bass foraging: evaluating potential match/mismatch regulation. Ecol.l Appl. **9**: 320–334.

Chick, J.H., and Van Den Avyle, M.J. 1999*b*. Effects of zooplankton spatial variation on growth of larval striped bass: an experimental approach. Trans. Am. Fish. Soc. **128**: 339–351.

Cowan, J.H., Jr., and Houde, E.D. 1992. Size-dependent predation on marine fish larvae by ctenophores, scyphomedusae and planktivorous fish. Fish. Oceanogr. **1**: 113–126.

Cowan, J.H., Jr., Houde, E.D., and Rose, K.A. 1996. Size-dependent vulnerability of marine fish larvae to predation: an individual-based numerical experiment. ICES J. Mar. Sci. **53**: 23–37.

Elliott, J.K., and Leggett, W.C. 1998. Larval condition and vulnerability to predation: an analysis based on mixed-prey experiments. Can. J. Fish. Aquat. Sci. **55**: 626–630.

Fuiman, L.A. 1993. Development of predator evasion in Atlantic herring, *Clupea harengus* L. Anim. Behav. **45**: 1101–1116.

Fuiman, L.A. 1994. The interplay of ontogeny and scaling in the interactions of fish larvae and their predators. J. Fish Biol. **45**(Suppl. A): 55–79.

Fuiman, L.A., and Gamble, J.C. 1989. Influence of experimental manipulations on predation of herring larvae by juvenile herring in large enclosures. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **191**: 359–365.

Fuiman, L.A., and Webb, P.W. 1988. Ontogeny of routine swimming activity and performance in zebra danios (Teleostei: Cyprinidae). Anim. Behav. **36**: 250–261.

Gerritsen, J., and Strickler, J.R. 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. J. Fish. Res. Board Can. **34**: 73–82.

Gotceitas, V., and Brown, J.A. 1993. Risk of predation to fish larvae in the presence of alternative prey: effects of prey size and number. Mar. Ecol. Prog. Ser. **98**: 215–222.

Grabe, S.A. 1996. Feeding chronology and habits of *Alosa* spp. (Clupeidae) juveniles form the lower Hudson River estuary, New York. Environ. Biol. Fishes, **47**: 321–326.

Hanson, J.M., and Qadri, S.U. 1984. Feeding ecology of age 0 pumpkinseed (*Lepomis gibbosus*) and black crappie (*Pomoxis nigromaculatus*) in the Ottawa River. Can. J. Zool. **62**: 613–621.

Higgs, D.M., and Fuiman, L.A. 1996. Ontogeny of visual and mechanosensory structure and function in Atlantic menhaden *Brevoortia tyrannus*. J. Exp. Biol. **199**: 2619–2629.

Janssen, J. 1982. Comparison of searching behavior for zooplankton in an obligate planktivore, blueback herring (*Alosa aestivalis*), and a facultative planktivore, bluegill (*Lepomis machrochirus*). Can. J. Fish. Aquat. Sci. **39**: 1649–1654.

Kean-Howie, J.C., Pearre, S., Jr., and Dickie, L.M. 1988. Experimental predation by sticklebacks on larval mackerel and protection of fish larvae by zooplankton alternative prey. Exp. Mar. Biol. Ecol. **124**: 239–259.

Laurence, G.C. 1972. Comparative swimming abilities of fed and starved larval largemouth bass (*Micropterus salmoides*). J. Fish Biol. **4**: 73–78.

Leggett, W.C., and Deblois, E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth. J. Sea Res. **32**: 119–134.

Letcher, B.H., Rice, J.A., Crowder, L.B., and Rose, K.A. 1996. Variability in survival of larval fish: disentangling components with a generalized individual-based model. Can. J. Fish. Aquat. Sci. **53**: 787–801.

Litvak, M.K., and Leggett, W.C. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. **81**: 13–24.

McGovern, J.C., and Olney, J.E. 1996. Factors affecting survival of early life stages and subsequent recruitment of striped bass on the Pamunkey River, VA. Can. J. Fish. Aquat. Sci. **53**: 1713–1726.

Miller, T.J., Crowder, L.B., Rice, J.A., and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657–1670.

Neilson, J.D., Perry, R.I., Valerio, P., and Waiwood, K.G. 1986. Condition of Atlantic cod *Gadus morhua* larvae after the transition to exogenous feeding: morphometrics, buoyancy and predator avoidance. Mar. Ecol. Prog. Ser. **32**: 229–235.

O'brien, W.J., Evans, B.I., and Browman, H.I. 1989. Flexible search tactics and efficient foraging in saltatory searching animals. Oecologia, **80**: 100–110.

Paradis, A.R., Pepin, P., and Brown, J.A. 1996. Vulnerability of fish eggs and larvae to predation: review of the influences of the relative size of prey and predator. Can. J. Fish. Aquat. Sci. **53**: 1226–1235.

Rice, J.A., Crowder, L.B., and Rose, K.A. 1993. Interactions between size-structured predator and prey populations: experimental test and model comparison. Trans. Am. Fish. Soc. **122**: 481–491.

Rosenthal, H., and Hempel, G. 1973. Experimental studies in feeding and food requirements of herring larvae (*Clupea harengus* L.). *In* Marine food chains. *Edited by* J.H. Steele. Oliver & Boyd, Edinburgh, U.K. pp. 344–364.

Rulifson, R.A., and Manooch, C.S., III. 1990. Recruitment of juvenile striped bass in the Roanoke River, North Carolina, as related to reservoir discharge. N. Am. J. Fish. Manage. **10**: 397–407.

Secor, D.H., Houde, E.D., and Monteleone, D.M. 1995. A mark–release experiment on larval striped bass, *Morone saxatilis*, in a Chesapeake Bay tributary. ICES. J. Mar. Sci. **52**: 87–101.

Yin, M.C., and Blaxter, J.H.S. 1987. Escape speeds of marine fish larvae during early development and starvation. Mar. Biol. **96**: 459–468.

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Recruitment variation related to fecundity in marine fishes

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Abstract: An understanding of the processes that control recruitment variation is central to explaining the population dynamics of fishes and predicting their responses to exploitation. Theory predicts that interannual variation in recruitment should be positively correlated with the fecundity of fish species, but empirical studies have not supported this hypothesis. Here, we adopt a phylogenetic comparative approach, which accounts for evolutionary relatedness among stocks and species, to investigate this relationship. We calculated the mean fecundity of fishes from 52 stocks at the mean length of maturity and related this to interannual recruitment variation. We found that in 13 of 14 comparisons between stocks or closely related species, the stocks with higher fecundity have higher recruitment variation. This was true whether or not we controlled for spawning stock size. However, when the analyses were repeated using a traditional cross-species approach, which did not account for the evolutionary relatedness of stocks, the relationships were not significant. This is the first empirical study to link fecundity with recruitment variation and suggests that fecundity is an important component of fish stock dynamics.

Résumé : Il est essentiel de comprendre les processus régissant la variation du recrutement pour expliquer la dynamique des populations de poissons et pour prévoir leurs réactions à l'exploitation. En théorie, la variation interannuelle du recrutement devrait être corrélée positivement avec la fécondité de l'espèce de poisson, mais cette hypothèse n'a pas été appuyée par des études empiriques. Dans le cadre de nos travaux, nous adoptons une approche phylogénétique comparative, qui prend en compte le rapprochement évolutionnaire entre les stocks et les espèces, afin d'en faire l'examen. Nous avons calculé pour des poissons provenant de 52 stocks la fécondité moyenne à la longueur moyenne de maturité, et nous avons lié cette valeur à la variation interannuelle du recrutement. Nos résultats montrent que, pour 13 des 14 comparaisons entre les stocks ou les espèces étroitement apparentés, la variation du recrutement est plus élevée chez les stocks à fécondité élevée. Cette constatation est vraie que nous contrôlions ou non la taille du stock de géniteurs. Toutefois, lorsque les analyses ont été reprises à l'aide de l'approche traditionnelle hétérospécifique, qui ne prenait pas en compte le rapprochement évolutionnaire des stocks, les rapports n'étaient pas significatifs. Il s'agit de la première étude empirique qui établit un lien entre la fécondité et la variation du recrutement, et qui fait ressortir que la fécondité serait une composante importante de la dynamique des stocks de poisson.

[Traduit par la Rédaction]

Introduction

Research into recruitment processes is central to understanding fish population dynamics and predicting responses to exploitation. Because year-class strength is largely determined during the early life history, attempts to explain recruitment variation have focused on processes that determine egg production and egg or larval mortality (e.g., Bailey and Houde 1989; Cushing 1996). These include density-dependent relationships between spawning stock and recruitment (Ricker 1954; Beverton and Holt 1957), predation on eggs and larvae (Bailey and Houde 1989; Chambers and Trippel 1997), and relationships between larval survival and the temporal (Cushing 1990) or spatial (Lasker 1978; Sinclair 1988) availability of suitable prey.

Interannual variation in recruitment varies greatly among stocks (Myers et al. 1995*a*; Myers and Barrowman 1996). Such patterns determine the susceptibility of stocks to intensive fishing at low population levels (Myers et al. 1995*b*, 1997) and the rates at which a stock can increase or decrease in abundance at a given level of fishing mortality. It has been suggested that stocks or species that exhibit the greatest recruitment variation should be the most fecund (Pepin and Myers 1991; Koslow 1992). Although this relationship has been shown for noctuid moths (Spitzer et al. 1984), empirical studies have failed to find such a relationship in fishes (Rothschild and DiNardo 1987; Mertz and Myers 1996). Fish studies have compared species with various reproductive strategies, including batch spawners and nonbatch spawners, and estimates of fecundity were not always possible at the same stages of the life history. In addition, these cross-species analyses did not account for the evolutionary relatedness between stocks or species, which may share life history characteristics due to common ancestry (Harvey and

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Pagel 1991). For example, herrings (*Clupea* spp.) share life history characteristics such as demersal spawning and pelagic feeding that may also be associated with recruitment variability. Thus, when these species are included in a cross-species analysis that involves a wide range of taxa, they do not represent statistically independent data points. A more powerful comparison is afforded by examining differences *between* stocks *within* species, analogous to the advantage of an experimental design that allows a paired *t* test rather than an unpaired test.

In this paper, we reexamine the evidence for relationships between fecundity and recruitment variation using a phylogenetic comparative approach that helps eliminate spurious correlations among life histories of related taxa. We calculate mean fecundity estimates for iteroparous species with determinate fecundity and relate these to estimates of recruitment variation.

Methods

Life history and recruitment data

We included all stocks of iteroparous marine fishes for which lengths (or ages) at maturity and length–fecundity relationships were known provided 10 or more consecutive years of recruitment data were available. Anadromous species were not included because of differences in reproductive biology, and batch spawners were not included because of difficulties in estimating annual fecundity.

Data were obtained for 13 intraspecific groups of stocks and one pair of species within a genus (*Brevoortia*) (Table 1). Relationships between stocks were determined from published genetic and morphometric evidence (e.g., Sakamoto 1984; Grant 1986; Dunn 1989; Nelson 1994). For eight species, data were only available for one stock. These species were included in the cross-species analysis but not in the comparative analyses. Atlantic cod stocks were split into east and west Atlantic groups on the basis of genetic evidence and treated separately (e.g., Bentzen et al. 1996 and references therein). Atlantic herring were considered genetically similar and therefore treated as one group (e.g., Turan et al. 1998 and references therein). Published relationships between length and fecundity were used to calculate mean fecundity at the size at which 50% of the stock attain maturity (L_{mat}), with five exceptions: for the two yellowtail flounder stocks and the northeast Arctic stocks of Atlantic cod and haddock, fecundity was calculated from the age at which 50% of the stock attain maturity (T_{mat}), and for the Strait of Georgia Pacific herring, a fecundity–weight relationship was used and L_{mat} converted to weight. If published L_{mat} estimates were not available, lengths were calculated from T_{mat}. We also calculated fecundity for each species at the theoretical asymptotic length using the von Bertalanffy growth parameter L_∞. The results of analyses using L_∞ as a comparable stage in the life history were consistent with those using L_{mat}, but they are not presented here because we consider fecundity at L_{mat} to be more robust when fecundity increases exponentially with body length. Imprecision in published estimates of L_{mat} may compound error in fecundity estimates, although these errors should be random with respect to the questions being asked. If possible, L_{mat} estimates were for females only. However, separate maturity data for females were not always available and combined estimates for both sexes had to be used.

Fecundity and L_{mat} estimates were taken from studies undertaken during time periods that matched the recruitment data as closely as possible. Appraisal of the dates for which these estimates were available does not indicate any obvious biases due to mismatches. This issue is explored further in the Results section.

Two estimates of recruitment variability were taken from Myers et al. (1995*a*) and Myers (1998): the standard deviation of ln-transformed recruitment estimates (SD) and, where available, the standard deviation of ln residuals from a Ricker spawner–recruitment function (Ricker SD). The SD measure is simply the recruitment variability over time. The Ricker SD measure gives an estimate of recruitment variability that is independent of spawning population size.

Phylogenetic comparative analyses

Differences in the values of fecundity, L_{mat}, and the two measures of recruitment variation (SD and Ricker SD) were calculated between pairs of related stocks or species. The resultant "contrasts" yield data that are statistically and phylogenetically independent, thereby offering finer resolution than traditional cross-species comparisons (Felsenstein 1985; Harvey and Pagel 1991). Where data for more than two stocks of a given species were available, all possible stock contrasts were calculated, and the mean of these contrasts was used in the analyses. Two analyses were performed: (*i*) testing the relationship between fecundity and recruitment variation and (*ii*) testing the relationship between body size (L_{mat}) and recruitment variation. In each case, binomial (one-tail) tests were used to test the prediction that stocks with greater fecundity or L_{mat} should exhibit greater recruitment variation. Fecundity and L_{mat} were $\log_{10}$ transformed before calculating the contrasts. We also conducted a comparative analysis with contrasts among species rather than among stocks. Mean values of recruitment, fecundity, and size at maturity were calculated for all stocks of each species, and contrasts calculated between pairs of related species (Fig. 1).

Cross-taxonomic analysis

We also performed a traditional cross-taxonomic analysis of the data that did not account for phylogenetic relatedness. Each stock was treated as an independent data point, and relationships between recruitment, fecundity, and size at maturity were determined using linear regressions.

Results

Phylogenetically based stock comparisons

The comparative analysis supports the hypothesis that stocks that exhibit high interannual recruitment variation (SD) have greater fecundity: in 13 of 14 comparisons, the stock with the higher fecundity has more variable recruitment. Thus, the data points (contrasts) in Fig. 2 are not distributed randomly above and below the horizontal line (binomial test, $n = 14$, $p < 0.001$). Note, however, that although the direction of the differences between stocks supports the hypothesis, the magnitude of the difference in fecundity was not correlated with the magnitude of the difference in recruitment variation (regression not forced through the origin: $F_{1,12} = 0.442$, $p = 0.519$). The only species that did not conform to the hypothesis was Atlantic herring.

The comparative analysis also indicates that high recruitment variation is associated with greater fecundity when we controlled for adult stock size (Ricker SD) (Fig. 2*b*) (binomial test: 13 positive contrasts, one negative contrast, $n = 14$, $p < 0.001$). As above, there was no significant relationship between the magnitude of the difference in fecundity and the magnitude of the difference in recruitment variation, which was slightly negative ($F_{1,11} = 2.654$, $p = 0.132$).

When body size was compared directly with recruitment variation, there was no significant relationship between L_{mat} and either SD (binomial test: 10 positive contrasts, four neg-

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Table 1. Species used in the analysis.

Species	Stock	L_{mat} (cm)	Years	Fecundity (1000s)	Years	Recruitment variation[a] SD	Ricker SD	Years	Reference[b] Maturity	Fecundity
Ammodytes marinus (sandeel)	Shetland	9.5	1970[c]	1.6	1985–1987	0.755	0.750	1976–1988	35	9
	S. North Sea	16.3	1970[c]	10.3	1961–1962	0.787	0.709	1976–1988	35	25
Anoplopoma fimbria (sablefish)	W. Canada	58.0	1979–1981	107.1	1979–1981	0.793	—	1972–1982	26	26
	W. United States	60.2	1986–1988	241.2	1986–1988	1.250	1.130	1977–1993	19	19
Brevoortia patronus (menhaden)	Gulf of Mexico	15.1	1960–1982	14.5	1981[c]	0.435	0.386	1964–1990	45	44
	U.S. Atlantic	20.6	1955–1981	51.4	1978–1981	0.528	0.598	1955–1994	47	46
Clupea harengus (Atlantic herring)	North Sea	25.7	1971[c]	41.7	1964–1965	0.858	0.610	1947–1987	21	21
	W. Scotland	27.0	—	39.5	1983–1984	0.572	0.511	1950–1988	21	21
	Downs	24.5	1961[c]	24.9	1954–1966	1.280	0.947	1923–1986	21	21
	S. Gulf of St. Lawrence (spring spawners)	30.0	1975[c]	59.7	1970–1972	0.806	—	1963–1984	21	21
	Iceland (spring spawners)	28.7*	1971[c]	30.2	1971[c]	2.330	1.010	1946–1967	36	36
	Iceland (summer spawners)	27.5*	1971[c]	73.6	1971[c]	0.919	0.720	1945–1993	36	36
	N. Irish Sea	23.7	1955–1970	31.5	1976	0.305	0.324	1972–1990	21	21
	Norway (spring spawners)	31.2	1973–1978	40.7	1971[c]	2.300	1.870	1950–1992	21	21
	SW Nova Scotia	31.0	1970–1972	92.1	1970–1972	0.857	0.859	1973–1983	27	28
Clupea pallasi (Pacific herring)	Central British Columbia	22.0	1970[c]	28.5	1974	0.803	0.747	1951–1988	49	11
	SE Alaska	25.0	1970[c]	37.6	1977[c]	1.020	1.040	1929–1963	49	11
	S. Strait of Georgia	17.9	1969–1980	12.2	1985	0.796	0.756	1951–1988	10	38
Gadus morhua (Atlantic cod)	Iceland	82.4*	1971[c]	2 418.0	1971[c]	0.447	0.435	1955–1992	36	36
	Irish Sea	60.9*	1970–1990	1 081.2	1995	0.534	0.5	1968–1994	22	2
	NE Arctic		1946–1982	3 660.0	1962–1979	0.746	0.651	1955–1990	20	1
	North Sea	69.7*	1970–1990	1 642.5	1971[c]	0.604	0.531	1963–1990	22	36
	Grand Bank	61.0	1975–1994	434.2	1964–1965	0.968	0.914	1959–1989	42	30
	Newfoundland	51.0	1982–1995	358.5	1966–1968	0.872	0.754	1962–1987	42	30
	St. Pierre Bank	58.3	1972–1994	370.9	1967–1970	0.368	0.342	1959–1989	42	30
Melanogrammus aeglefinus (haddock)	Grand Bank	42.8*	1947–1966	302.4	1957–1961	2.070	—	1942–1966	39	14
	NE Arctic	52.0*	1962–1988	960.0	1968–1971	1.350	1.230	1949–1993	24	5
	N. North Sea	31.5*	1976–1979	120.2	1976–1979	1.110	1.170	1961–1991	13	13
	SW Scotian Shelf	43.0*	1970–1985	278.7	1970–1985	0.792	0.778	1962–1985	48	48
Merlangius merlangus (whiting)	North Sea	20.2*	1970–1990	107.7	1964–1969	0.506	0.505	1965–1991	22	12
	W. Scotland	28.4*	1970–1990	259.0	1970	0.664	0.635	1965–1991	22	12
Pleuronectes ferruginae (yellowtail flounder)	Grand Bank	35.4	1965–1972	737.2	1966–1967	0.587	0.563	1968–1982	34	33
	S. New England	30.4	1954[c]	787.8	1976	1.060	0.983	1973–1992	34	18
Pleuronectes platessa (plaice)	Celtic Sea	28.8*	1970–1990	59.6	1990	0.509	0.516	1977–1990	22	17
	Irish Sea	26.2*	1970–1977	46.3	1987–1988	0.302	0.323	1964–1991	4	15
	North Sea	26.6*	1970–1990	40.0	1977–1980	0.411	0.388	1957–1991	22	15

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Table 1 (*concluded*).

Species	Stock	L_{mat} (cm)	Years	Fecundity (1000s)	Years	Recruitment variation[a] SD	Ricker SD	Years	Reference[b] Maturity	Fecundity
Scomber scombrus (Atlantic mackerel)	NW Atlantic	28.0*	1970[c]	144.5	1983–1984	1.220	1.180	1972–1990	49	29
	W. Europe	26.2*	1970–1990	104.3	1986	0.639	0.638	1962–1989	22	22
Solea solea (sole)	Celtic Sea	24.2*	1970–1990	44.7	1988	0.568	0.474	1972–1992	22	16
	North Sea	24.8*	1970–1990	86.1	1991	0.807	0.809	1957–1989	22	51
Theragra chalcogramma (walleye pollock)	E. Bering Sea	34.0*	1979[c]	58.1	1979[c]	0.407	0.405	1964–1987	3	3
	SE Hokkaido	35.0*	1959[c]	158.6	1959[c]	0.473	0.477	1971–1985	3	3
	SE Kamchatka	37.0*	1986[c]	130.6	1986[c]	0.544	0.436	1970–1981	3	3
	W. Bering Sea	42.0*	1971–1983	126.8	1971–1983	0.395	0.295	1966–1990	3	3
Gadus macrocephalus (Pacific cod)	Hectate Strait	50.3*	1986	679.5	1990	0.446	0.423	1968–1986	50	43
Hippoglossoides platessoides (American plaice)	Grand Bank	47.7*	1957–1963	441.8	1957–1962	0.207	0.159	1965–1983	32	31
Micromesistius poutassou (blue whiting)	N. Europe	25.1*	1970–1990	24.4	1968–1969	0.603	0.568	1970–1989	22	22
Platichthys flesus (European flounder)	SW Baltic	22.6*	1993–1995	397	1993–1995	0.245	0.247	1969–1990	23	23
Limanda aspera (yellowfin sole)	W. Kamchatka	28.4*	1961–1969	581.7	1963–1969	0.647	0.523	1968–1981	41	40
Reinhardtius hippoglossoides (Greenland halibut)	NW Atlantic	71.2*	1983[c]	32.1	1992[c]	0.235	0.151	1969–1982	6	37
Scomberomorus cavalla (king mackerel)	W. Gulf of Mexico	47.5*	1986[c]	159.8	1986[c]	1.450	1.470	1962–1989	8	8
Trisopterus esmarkii (Norway pout)	North Sea	18.6*	1970–1990	31.3	1983[c]	0.559	0.525	1978–1992	22	7

Note: Taxa are grouped to show comparisons, except for the single stocks, which were paired according to phylogenetic relatedness (Fig.1). Column headings are as follows: L_{mat} (length at maturity), fecundity (number of eggs per year calculated at L_{mat} or age at maturity), SD (SD of ln recruitment), and Ricker SD (SD of ln Ricker residuals controlling for adult stock size). Lengths are for males and females combined except where marked with an asterisk, which indicates data for females only.

[a]Myers et al. (1995*a*) and Myers (1998).

[b]1. Aldonov et al. (1982), 2. Anonymous (1997), 3. Balykin (1986), 4. Basmi and Grove (1985), 5. Blinov (1990), 6. Bowering (1983), 7. Dann (1983), 8. Finucane et al. (1986), 9. Gauld and Hutcheon (1990), 10. Haist and Stocker (1985), 11. Hay (1985), 12. Hislop and Hall (1974), 13. Hislop and Shanks (1979), 14. Hodder (1963), 15. Horwood (1990), 16. Horwood (1993*a*), 17. Horwood (1993*b*), 18. Howell and Kesler (1977), 19. Hunter et al. (1989), 20. Hylen and Rorvik (1984), 21. Jennings and Beverton (1991), 22. Jennings et al. (1998), 23. Kosior et al. (1996), 24. Kovtsova (1993), 25. Macer (1966), 26. Mason et al. (1983), 27. Messieh (1976), 28. Messieh et al. (1985), 29. Neja (1992), 30. Pinhorn (1984), 31. Pitt (1963), 32. Pitt (1966), 33. Pitt (1971), 34. Pitt (1974), 35. calculated from Reay (1970), 36. Schopka and Hempel (1973), 37. Serebryakov et al. (1992), 38. Tanasichuk et al. (1993), 39. Templeman et al. (1978), 40. Tikhonov (1977), 41. Tikhonov (1983), 42. Trippel et al. (1997), 43. Tyler (1995), 44. Vaughan (1987), 45. calculated from Vaughan (1987), 46. Vaughan and Smith (1988), 47. calculated from Vaughan and Smith (1988), 48. Waiwood and Buzeta (1989), 49. Ware (1980), 50. Welch and Foucher (1988), 51. Witthames et al. (1995).

[c]Year refers to publication of original reference. Otherwise, the years refer to when data were collected.

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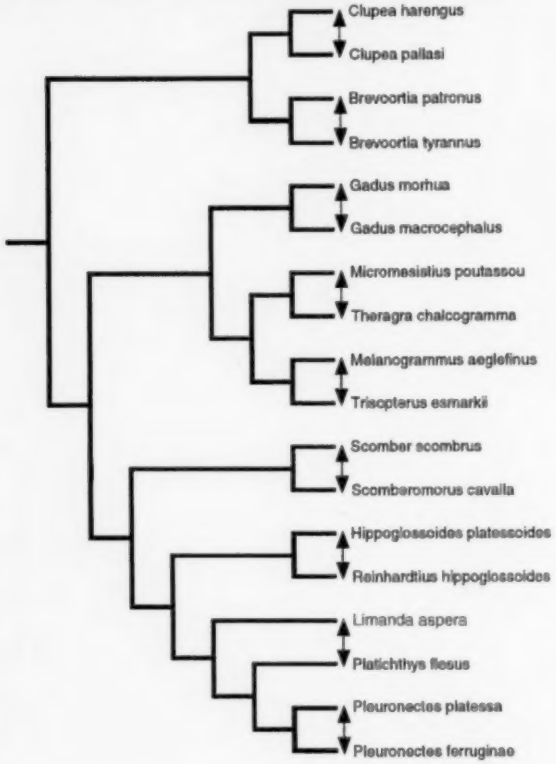
Fig. 1. Reconstructed phylogeny of marine fish species used in "species comparison" analysis showing pairs of closely related species.



ative contrasts, $n = 14$, $p = 0.090$) or Ricker SD (seven positive contrasts, six negative contrasts).

Temporal biases?

Life history parameters of fish stocks usually change over time. Any such changes, however, are unlikely to bias the results of these analyses. First, consider fecundity. Unfortunately, a time series of data on fecundity was available only for North Sea whiting (Hislop and Hall 1974). There was a significant reduction in fecundity from 1964 to 1969 ($r^2 = 0.82$, $n = 5$, $p = 0.034$). This time series overlapped the early data for recruitment variation (Table 1), and this stock had less recruitment variation than its sister stock with which it was compared (West Scotland whiting) (Table 1). Therefore, if North Sea whiting fecundity continued to decline through the period when most of the recruitment data were available, this would increase the magnitude of the difference between the stocks in the direction predicted by the hypothesis, and the number of positive and negative contrasts would remain unchanged.

We also investigated potential biases due to temporal changes in length at maturity. Of the four stocks in Table 1 for which 4 or more years of data were available, one, the Grand Bank Atlantic cod, showed a significant decline in length at maturity ($r^2 = 0.94$, $n = 4$, $p = 0.028$). Again, this does not affect the results presented above. First, the other two western Atlantic cod stocks showed similar, although nonsignificant, declines, and more important, the time series for recruitment matched each other closely (Table 1). Second, to ensure that the final result would not be affected by these declines, we performed an additional analysis whereby fecundities of all three western Atlantic cod stocks were recalculated for age at maturity data taken from the same time period (1980–1995). Once again, the mean pairwise differences between the three stocks increase in the direction predicted by the hypothesis, and the count of comparisons for and against the hypothesis (13:1) remains unchanged.

Phylogenetically based species comparisons

We calculated mean values for each species based on their constituent stocks' recruitment, fecundity, and maturity data. Comparisons were then made between closely related species (Fig. 1). At this higher level of analysis, the sample size was smaller, and the relationship described above between fecundity and recruitment variation was not significant (binomial tests for both SD and Ricker SD: seven positive contrasts, two negative contrasts, $n = 9$, $p = 0.090$). Comparison between body size and each measure of recruitment variation produced the same result (seven positive contrasts, two negative contrasts).

Cross-taxonomic analysis

Traditional cross-taxonomic analyses, where phylogenetic relationships are ignored, failed to reveal any relationship between fecundity and either measure of recruitment variation, despite the larger sample sizes (Fig. 3) (SD, $F_{50,1} = 0.150$, $p = 0.701$; Ricker SD, $F_{47,1} = 0.285$, $p = 0.596$). There was also no direct relationship between body size (L_{mat}) and recruitment variation (SD, $F_{47,1} = 0.002$, $p = 0.964$; Ricker SD, $F_{45,1} = 0.918$, $p = 0.343$).

Discussion

This is the first study to demonstrate that fish stocks with higher fecundity have more variable recruitment. This association was revealed using phylogenetically independent comparisons, largely at the level of stocks within species. Although the direction of the relationship was toward higher recruitment variation in the stock (or species) with the highest fecundity in 13 of the 14 taxa examined, the *magnitude* of this difference was not correlated with the magnitude of the difference in fecundity. Thus, although knowledge of fecundity allows accurate prediction of which stock within species will have higher recruitment variation, it has no predictive power for exactly how high that recruitment variation will be. Note, too, that the pattern could not be detected using a traditional cross-taxonomic analysis, in accordance with previous studies (Rothschild and DiNardo 1987; Mertz and Myers 1996).

The advantage of making phylogenetic comparisons between related taxa is that any relationships between variables are less confounded by other differences among taxa (Harvey and Pagel 1991). Even closely related species can have quite different life histories, which are likely to influence fecundity and (or) recruitment. These variable traits could have obscured any general relationship between fecundity and recruitment. Indeed, comparison between the results of phylogenetically based analyses and nonphylogenetically

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Fig. 2. Relationship between phylogenetically independent contrasts in log fecundity and interannual recruitment variation of marine fish species. The null expectation is for equal numbers of data points falling above and below the horizontal line. (*a*) SD of ln recruitment; (*b*) SD of ln Ricker residuals of recruitment, controlling for adult stock size.

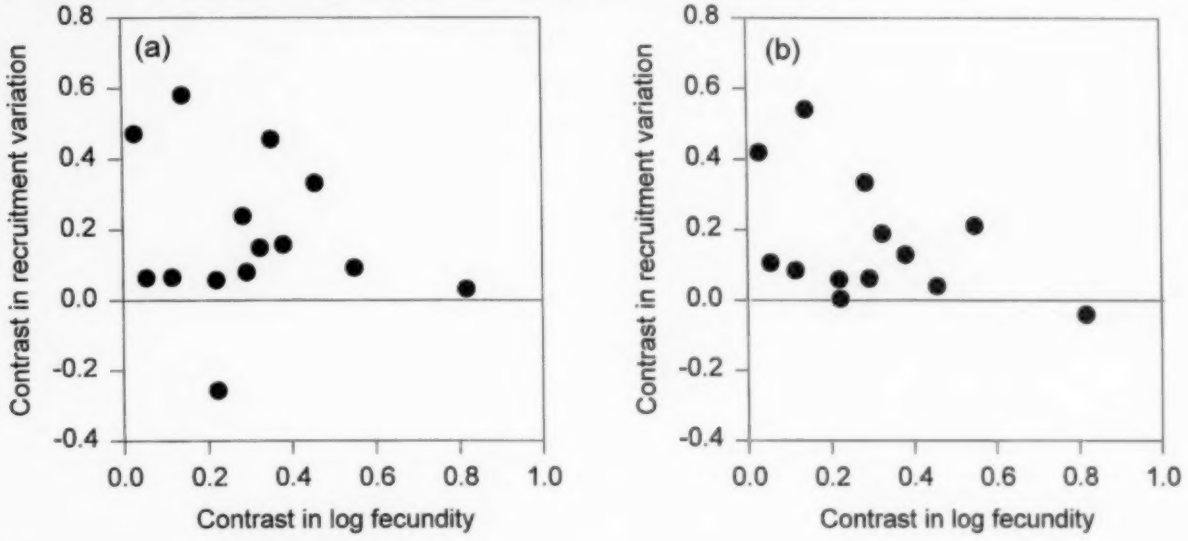
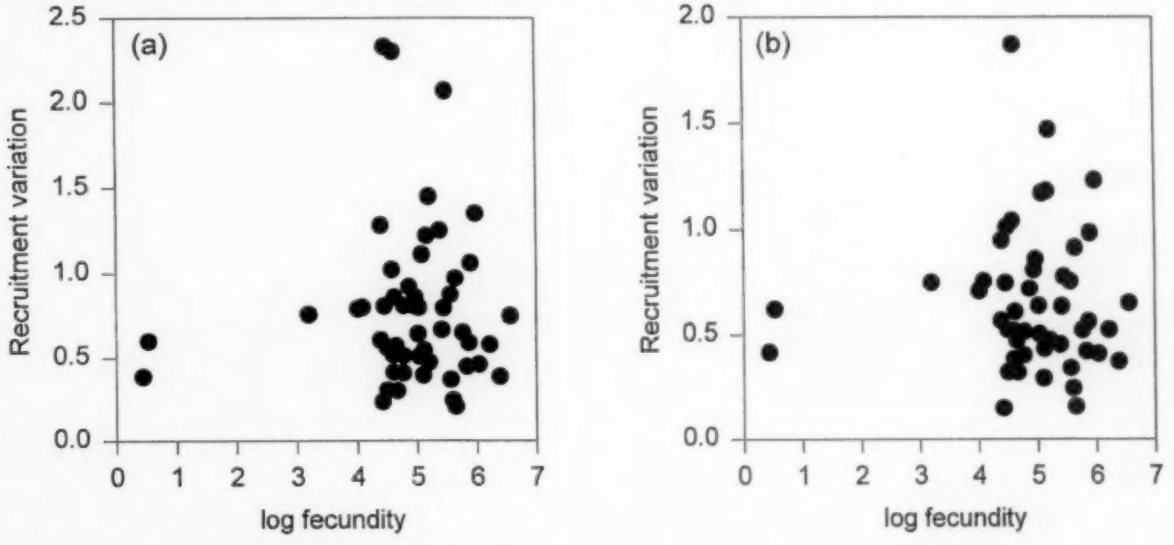


Fig. 3. Cross-taxonomic relationship between log fecundity and interannual recruitment variation of marine fish species. (*a*) SD of ln recruitment; (*b*) SD of ln Ricker residuals of recruitment, controlling for adult stock size.



based analyses confirms the value of this technique, which has recently been questioned for birds and mammals (Ricklefs and Starck 1996). Furthermore, a recent study on marine taxa showed that the phylogenetic method can detect relationships between aspects of life histories and abundance trends in northeast Atlantic fish stocks (Jennings et al. 1998). These relationships could not be detected using traditional cross-taxonomic analyses.

The estimates of recruitment variation are calculated from the best available spawner and recruitment data (Myers et al. 1995*a*; Myers 1998). However, these estimates are not entirely satisfactory. Recruitment indices are never perfect, and the SD estimate may simply reflect variation in spawner abundance. The estimate that controls for spawner abundance is based on residuals from a fitted Ricker spawner–recruit model, but selection of an appropriate model for such relationships is a notorious problem in fisheries science (Koslow 1992). However, the consistency between the two measures lends some credence to the results (see also Mertz and Myers 1996).

Marine fishes show an inverse relationship between fecundity and egg size (Duarte and Alcaraz 1989; Elgar 1990). As

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such, species with smaller eggs and larvae may exhibit greater recruitment variation. While relationships between these variables have not been found in traditional cross-species analyses (Pepin and Myers 1991), a phylogenetically explicit analysis may tease out any relationship.

Individual changes in quantity and quality of eggs produced contribute to variation in the relationship between parent stock and subsequent recruitment (Rijnsdorp 1991). Although the main density-dependent regulatory processes operate during the early life history (e.g., Bailey and Houde 1989), in a heavily fished stock, density-dependent processes in the adult phase may lead to increases in mean individual fecundity and hence recruitment variability. This, coupled with a truncated age structure, would lead to greater interannual instability in stock size, making it more difficult to manage effectively (e.g., Beverton 1990).

Our results demonstrate the strength of the phylogenetic comparative method, which could help to identify other life history traits and environmental variables that affect recruitment variation, after relationships with fecundity have been accounted for. Many environmental factors can affect survival at various stages in the early life history (e.g., Lasker 1978; Bradford and Cabana 1997). Uncertainty about what regulates recruitment is due to a dearth of relevant information, not only about the fish themselves, but also about the environment in which they live (Neill et al. 1994). Greater understanding of the biology and ecology of marine fish is required before the complex issue of recruitment variability can be resolved.

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References

Aldonov, V.K., Borisov, V.M., and Serebriakov, V.P. 1982. Fecundity of Arcto-Norwegian cod spawning populations. ICES C.M. 1982/G:19.

Anonymous. 1997. Estimation of fish biomass in the Irish Sea by means of the annual egg production method. Ministry of Agriculture, Fisheries and Food, Lowestoft, U.K.

Bailey, K.M., and Houde, E.D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Balykin, P.A. 1986. Fecundity of walleye pollock, *Theragra chalcogramma*, in the western part of the Bering Sea. Vopr. Ikhtiol. **1**: 164–168.

Basmi, R.A., and Grove, D.J. 1985. Studies on feeding, growth and production of a recruited inshore population of *Pleuronectes platessa* (L.) at East Anglesey, North Wales. J. Fish Biol. **27**: 765–783.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**: 2706–2721.

Beverton, R.J.H. 1990. Small marine pelagic fish and the threat of fishing: are they endangered? J. Fish Biol. **37**(Suppl. 1): 5–16.

Beverton, R.J.H., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Fish. Invest. Ser. II Mar. Fish. G.B. Minist. Agric. Fish. Food No. 19.

Blinov, V.V. 1990. Reproduction and survival of recruitment of Arcto-Norwegian haddock considering on the base of the catastrophe theory. ICES C.M. 1990/D:4.

Bowering, W.R. 1983. Age, growth, and sexual maturity of Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in the Canadian northwest Atlantic. Fish. Bull. U.S. **81**: 599–611.

Bradford, M.J., and Cabana, G. 1997. Interannual variability in stage-specific survival rates and the causes of recruitment variation. *In* Early life history and recruitment in fish populations. *Edited by* C. Chambers and E.A. Trippel. Chapman and Hall, London, U.K. pp. 469–494.

Chambers, C., and Trippel, E.A. (*Editors*). 1997. Early life history and recruitment in fish populations. Chapman and Hall, London, U.K.

Cushing, D.H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv. Mar. Biol. **26**: 249–293.

Cushing, D.H. 1996. Towards a science of recruitment in fish populations. Ecology Institute, Oldendorf/Luhe.

Dann, J. 1983. Fecundity estimates of Norway pout (*Trisopterus esmarkii*) taken on ICES young fish surveys in 1979 and 1980. ICES C.M. 1983/G:5.

Duarte, C.M., and Alcaraz, M. 1989. To produce many small or few large eggs: a size dependent reproductive tactic of fish. Oecologia, **80**: 401–404.

Dunn, J.R. 1989. A provisional phylogeny of gadid fishes based on adult and early life-history characters. *In* Papers on the systematics of gadiform fishes. *Edited by* D.M. Cohen. Nat. Hist. Mus. Los Angel Cty. Sci. Ser. **32**: 209–235.

Elgar, M.A. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. Oikos, **59**: 283–287.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. **125**: 1–15.

Finucane, J.H., Collins, L.A., Brusher, H.A., and Saloman, C.H. 1986. Reproductive biology of king mackerel, *Scomberomorus cavalla*, from the southeastern United States. Fish. Bull. U.S. **84**: 841–850.

Gauld, F.A., and Hutcheon, J.R. 1990. Spawning and fecundity in the lesser sandeel, *Ammodytes marinus* Raitt, in the northwestern North Sea. J. Fish Biol. **36**: 611–613.

Grant, W.S. 1986. Biochemical genetic divergence between Atlantic, *Clupea harengus*, and Pacific, *Clupea pallasi*, herring. Copeia, **1986**: 714–719.

Haist, V., and Stocker, M. 1985. Growth and maturation of Pacific herring (*Clupea harengus*) in the Strait of Georgia. Can. J. Fish. Aquat. Sci. **42**: 138–146.

Harvey, P.H., and Pagel, M.D. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, U.K.

Hay, D.E. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. **42**: 111–126.

Hislop, J.R.G., and Hall, W.B. 1974. The fecundity of whiting *Merlangius merlangus* (L.) in the North Sea, the Minch and in Iceland. J. Cons. Int. Explor. Mer, **36**: 42–49.

Hislop, J.R.G., and Shanks, A.M. 1979. Recent investigations on the reproductive biology of the haddock, *Melanogrammus aeglefinus*, of the northern North Sea and the effects on fecundity of infection with the copepod parasite *Lernacocera branchialis*. J. Cons. Int. Explor. Mer, **39**: 244–251.

Hodder, V.M. 1963. Fecundity of Grand Bank haddock. J. Fish. Res. Board Can. **20**: 1465–1487.

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Horwood, J.W. 1990. Fecundity and maturity of plaice (*Pleuronectes platessa*) from Cardigan Bay. J. Mar. Biol. Assoc. U.K. **70**: 515–529.

Horwood, J.W. 1993*a*. The Bristol Channel sole (*Solea solea* (L.)). Adv. Mar. Biol. **29**: 215–367.

Horwood, J.W. 1993*b*. Fecundity and biomass of plaice (*Pleuronectes platessa* L.) in the northern Celtic Sea. ICES J. Mar. Sci. **50**: 315–323.

Howell, W.H., and Kesler, D.H. 1977. Fecundity of the southern New England stock of yellowtail flounder, *Limanda ferruginea*. Fish. Bull. U.S. **75**: 877–880.

Hunter, J.R., Macewicz, B.J., and Kimbrell, C.A. 1989. Fecundity and other aspects of the reproduction of sablefish, *Anoplopoma fimbria*, in central California waters. Calif. Coop. Oceanic Fish. Invest. Rep. **30**: 61–72.

Hylen, A., and Rorvik, C.J. 1984. Estimating the maturity ogive for the northeast Arctic cod by a modified assessment model. *In* Proceedings of the Soviet–Norwegian Symposium on Reproduction and Recruitment of Arctic Cod. *Edited by* O.R. Godoe and A. Tilseth. Institute of Marine Research, Bergen, Norway. pp. 154–188.

Jennings, S., and Beverton, R.J.H. 1991. Intraspecific variation in the life history tactics of Atlantic herring (*Clupea harengus* L.) stocks. ICES J. Mar. Sci. **48**: 117–125.

Jennings, S., Reynolds, J.D., and Mills, S.C. 1998. Life history correlates of responses to fisheries exploitation. Proc. R. Soc. Lond. Ser. B, Biol. Sci. **265**: 333–339.

Kosior, M., Kuczynski, J., and Grygiel, W. 1996. Reproduction of Baltic flounder (*Platichthys flesus* L.) in relation to some somatic factors. ICES C.M. 1996/J:29.

Koslow, J.A. 1992. Fecundity and the stock–recruitment relationship. Can. J. Fish. Aquat. Sci. **49**: 210–217.

Kovtsova, M.V. 1993. Growth and maturation of Arcto-Norwegian haddock in 1987–1990. ICES C.M. 1993/G:58.

Lasker, R. 1978. The relationship between oceanographic conditions and larval anchovy food in the California current: identification of factors contributing to recruitment failure. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **173**: 212–230.

Macer, C.T. 1966. Sandeels (Ammodytidae) in the southwestern North Sea; their biology and fishery. Fish. Invest. Ser. II Mar. Fish. G.B. Minist. Agric. Fish. Food No. 24.

Mason, J.C., Beamish, R.J., and McFarlane, G.A. 1983. Sexual maturity, fecundity, spawning, and early life history of sablefish (*Anoploma fimbria*) off the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. **40**: 2126–2134.

Mertz, G., and Myers, R.A. 1996. Influence of fecundity on recruitment variability of marine fish. Can. J. Fish. Aquat. Sci. **53**: 1618–1625.

Messieh, S. 1976. Fecundity studies on Atlantic herring from the southern Gulf of St. Lawrence and along the Nova Scotia coast. Trans. Am. Fish. Soc. **3**: 384–395.

Messieh, S., Anthony, V., and Sinclair, M. 1985. Fecundities of Atlantic herring populations in the northwest Atlantic. ICES C.M. 1985/H:8.

Myers, R.A. 1998. Ransom Myers' stock recruitment database. Internet site: www.mscs.dal.ca/~myers/welcome.html

Myers, R.A., and Barrowman, N.J. 1996. Is fish recruitment related to spawner abundance? Fish. Bull. U.S. **94**: 707–724.

Myers, R.A., Bridson, J., and Barrowman, N.J. 1995*a*. Summary of worldwide spawner and recruitment data. Can. Tech. Rep. Fish. Aquat. Sci. No. 2024.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., and Rosenberg, A.A. 1995*b*. Population dynamics of exploited fish stocks at low population levels. Science (Washington, D.C.), **269**: 1106–1108.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol. Appl. **7**: 91–106.

Neill, W.H., Miller, J.M., van der Veer, H.E., and Winemiller, K.O. 1994. Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. Neth. J. Sea Res. **32**: 135–152.

Neja, Z. 1992. Maturation and fecundity of mackerel (*Scomber scombrus* L.) in northwest Atlantic. Acta Ichthyol. Pisc. **22**: 125–140.

Nelson, J.S. 1994. Fishes of the world. John Wiley & Sons, New York.

Pepin, P., and Myers, R.A. 1991. Significance of egg and larval size to recruitment variability of temperate marine fish. Can. J. Fish. Aquat. Sci. **48**: 1820–1828.

Pinhorn, A.T. 1984. Temporal and spatial variation in fecundity of Atlantic cod (*Gadus morhua*) in Newfoundland waters. J. Northwest Atl. Fish. Sci. **5**: 161–170.

Pitt, T.K. 1963. Fecundity of the American plaice, *Hippoglossoides platessoides*, from Grand Bank and Newfoundland areas. J. Fish. Res. Board Can. **20**: 597–613.

Pitt, T.K. 1966. Sexual maturity and spawning of the American plaice, *Hippoglossoides platessoides* (Fabricius), from Newfoundland and Grand Bank areas. J. Fish. Res. Board Can. **23**: 651–672.

Pitt, T.K. 1971. Fecundity of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank, Newfoundland. J. Fish. Res. Board Can. **28**: 456–457.

Pitt, T.K. 1974. Age composition and growth of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank. J. Fish. Res. Board Can. **31**: 1800–1802.

Reay, P.J. 1970. Synopsis of biological data on North Atlantic sandeels of the genus *Ammodytes* (*A. tobianus*, *A. dubius*, *A. americanus*, *A. marinus*). FAO Fish. Synop. No. 82.

Ricker, W.E. 1954. Stock and recruitment. J. Fish. Res. Board Can. **11**: 559–623.

Ricklefs, R.E., and Starck, J.M. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. Oikos, **77**: 167–172.

Rijnsdorp, A.D. 1991. Reproductive variability in North Sea plaice, sole and cod. J. Cons. Cons. Int. Explor. Mer, **47**: 352–375.

Rothschild, B.J., and DiNardo, G.T. 1987. Comparison of recruitment variability and life history data among marine and anadromous fishes. Am. Fish. Soc. Symp. **1**: 531–546.

Sakamoto, K. 1984. Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes). Mem. Fac. Fish. Hokkaido Univ. **36**: 95–215.

Schopka, S.A., and Hempel, G. 1973. The spawning potential of populations of herring (*Clupea harengus* L.) and cod (*Gadus morhua*) in relation to the rate of exploitation. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **164**: 178–185.

Serebryakov, V.P., Chumakov, A.K., and Tevs, I.I. 1992. Spawning stock, population fecundity and year-class strength of Greenland halibut (*Reinhardtius hippoglossoides*) in the northwest Atlantic. J. Northwest Atl. Fish. Sci. **14**: 107–113.

Sinclair, M. 1988. Marine populations: an essay on population regulation, and speciation. University of Washington Press, Seattle, Wash.

Spitzer, K., Rejmánek, M., and Soldán, T. 1984. The fecundity and long-term variability in abundance of noctuid moths (Lepidoptera, Noctuidae). Oecologia, **62**: 91–93.

Tanasichuk, R.W., Kristofferson, A.H., and Gillman, D.V. 1993. Comparison of some life history characteristics of Pacific her-

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ring (*Clupea pallasi*) from the Canadian Pacific Ocean. Can. J. Fish. Aquat. Sci. **50**: 964–971.

Templeman, W., Hodder, V.M., and Wells, R. 1978. Sexual maturity and spawning in haddock, *Melanogrammus aeglefinus*, of the southern Grand Bank. Int. Comm. Northwest Atl. Fish. Res. Bull. **13**: 53–65.

Tikhonov, V.I. 1977. Changes in fecundity and rate of maturation of the yellowfin sole. Sov. J. Mar. Biol. **3**: 214–218.

Tikhonov, V.I. 1983. Fecundity of flounder on the west Kamchatka shelf. Sov. J. Mar. Biol. **8**: 136–140.

Trippel, E.A., Morgan, M.J., Frechet, A., Rollet, C., Sinclair, A., Annand, C., Beanlands, D., and Brown, L. 1997. Changes in age and length at sexual maturity of northwest Atlantic cod, haddock and pollock stocks, 1972–1995. Can. Tech. Rep. Fish. Aquat. Sci. No. 2157.

Turan, C., Carvalho, G.R., and Mork, J. 1998. Molecular genetic analysis of Atlanto-Scandian herring (*Clupea harengus*) populations using allozymes and mitochondrial markers. J. Mar. Biol. Assoc. U.K. **78**: 269–283.

Tyler, A.V. 1995. Warmwater and cool-water stocks of Pacific cod (*Gadus macrocephalus*): a comparative study of reproductive biology and stock dynamics. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 537–545.

Vaughan, D.S. 1987. Stock assessment of the Gulf menhaden, *Brevoortia patronus*, fishery. NOAA Tech. Rep. NMFS No. 58.

Vaughan, D.S., and Smith, J.W. 1988. Stock assessment of the Atlantic menhaden, *Brevoortia tyrannus*, fishery. NOAA Tech. Rep. NMFS No. 63.

Waiwood, K.G., and Buzeta, M.I. 1989. Reproductive biology of southwest Scotian shelf haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. **46**: 153–170.

Ware, D.M. 1980. Bioenergetics of stock and recruitment. Can. J. Fish. Aquat. Sci. **37**: 1012–1024.

Welch, D.W., and Foucher, R.P. 1988. A maximum likelihood methodology for estimating length-at-maturity with application to Pacific cod (*Gadus macrocephalus*) population dynamics. Can. J. Fish. Aquat. Sci. **45**: 333–343.

Witthames, P.R., Greer-Walker, M., Dinis, M.T., and Whiting, C.L. 1995. The geographical variation in the potential annual fecundity of Dover sole, *Solea solea* (L.), from European shelf waters during 1991. Neth. J. Sea Res. **34**: 45–58.

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Impact of $Ca(OH)_2$ treatment on macroinvertebrate communities in eutrophic hardwater lakes in the Boreal Plain region of Alberta: in situ and laboratory experiments

K.A. Yee, E.E. Prepas, P.A. Chambers, J.M. Culp, and G. Scrimgeour

Abstract: $Ca(OH)_2$ treatment has been recently used to remediate water quality problems associated with eutrophication in lakes on the Boreal Plain of western Canada. This study examines how $Ca(OH)_2$ treatment affects the survival of *Hyalella azteca* and larvae of *Chironomus* spp. in laboratory microcosms and the abundance and seasonal dynamics of macroinvertebrates in two eutrophic hardwater lakes. Macroinvertebrate data for three reference lakes sampled during the same time period are also presented. Additions of 225 and 295 g $Ca(OH)_2 \cdot m^{-2}$ (74 and 107 $mg \cdot L^{-1}$) to two lakes resulted in lake water pH remaining within its natural range (<10) and had no discernable effects on macroinvertebrate density or biomass up to 2 years after treatment. Macroinvertebrate response to $Ca(OH)_2$ in hardwater lakes differed markedly from results reported for softwater lakes. After 4 days, no detectable influence was observed on survivorship of *Chironomus* spp. with laboratory dosages of 0–300 g $Ca(OH)_2 \cdot m^{-2}$ (0–1781 $mg \cdot L^{-1}$), but survivorship of *H. azteca* was reduced by ≥50% at dosages that raised water pH to ≥10 (300 g $Ca(OH)_2 \cdot m^{-2}$). The sensitivity of *H. azteca*, but not *Chironomus* spp., could be a result of singular or combined effects of $Ca(OH)_2$ levels, precipitate, or changes in pH. It may also reflect the greater dependence of *H. azteca* than *Chironomus* spp on the water above, rather than below, the sediment–water interface.

Résumé : Un traitement au $Ca(OH)_2$ a été utilisé dernièrement pour corriger les problèmes de qualité de l'eau associés à l'eutrophisation des lacs de la Plaine boréale du Canada. La présente étude porte sur la façon dont le traitement au $Ca(OH)_2$ influe sur la survie des larves d'*Hyalella azteca* et de *Chironomus* spp. dans des microcosmes en laboratoire, de même que sur l'abondance et la dynamique saisonnière des macroinvertébrés dans deux lacs d'eau dure eutrophes. Suite à l'addition de 225 et de 295 $g \cdot m^{-2}$ (74 et 107 $mg \cdot L^{-1}$) de $Ca(OH)_2$, le pH de l'eau du lac est demeuré dans sa fourchette de valeurs naturelle (<10). Deux ans après le traitement, on n'observait aucun effet visible du $Ca(OH)_2$ sur la densité ou la biomasse des macroinvertébrés. La réaction des macroinvertébrés au $Ca(OH)_2$ dans les lacs d'eau dure différait énormément des résultats signalés dans le cas des lacs d'eau douce. Après 4 jours, l'administration de doses expérimentales comprises entre 0 et 300 $g \cdot m^{-2}$ de $Ca(OH)_2$ (0 à 1 781 $mg \cdot L^{-1}$) n'avait aucune influence sur la survie des larves de *Chironomus* spp., mais la survie de *H. azteca* a été réduite de ≥50% avec des doses qui élèvent le pH de l'eau à ≥10 [300 $g \cdot m^{-2}$ de $Ca(OH)_2$]. La sensibilité de *H. azteca*, mais pas de *Chironomus* spp., pourrait être attribuable à des effets simples ou combinés des concentrations de $Ca(OH)_2$, du précipité ou des variations de pH. Elle peut aussi refléter le fait que *H. azteca* est plus dépendante que *Chironomus* spp. de la couche d'eau située au-dessus de l'interface sédiment–eau que de l'eau sous l'interface.

[Traduit par la Rédaction]

Introduction

The Boreal Plain region of central Alberta contains many eutrophic to hypereutrophic hardwater lakes with high internal sediment phosphorus (P) loading rates (Mitchell and Prepas 1990). The majority of these lakes experience extensive blue-green algal blooms (Kotak et al. 1993; Zhang and Prepas 1996*a*) that may compromise water quality and lead to hypolimnetic dissolved oxygen (DO) depletion when algae decompose. The distribution, growth, and life cycles of aerobic organisms such as benthic macroinvertebrates may then be limited by DO availability (Brinkhurst 1974; Jónasson 1978).

Lime ($Ca(OH)_2$ and (or) $CaCO_3$) additions have been used during the past 20 years to increase the alkalinity and pH of acidified lakes in eastern Canada and Europe (Olem et al. 1991). More recently, lime has been tested as a means of inactivating P in eutrophic hard waters (Prepas et al. 1990; Babin et al. 1994). These studies showed that multiple successive additions of lime to the open water were sufficient to

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Table 1. Physical and chemical (1991 means) characteristics of the five study lakes.

	Treated		Reference		
Characteristic	Lofty	North Halfmoon	Jenkins	Crooked[a]	Baptiste[a]
Latitude (N)	54°44′	54°04′	54°55′	54°55′	54°45′
Longitude (W)	112°29′	113°21′	113°36′	113°32′	113°33′
Surface area (km^2)	0.7	0.8	1.6	2.4	4.7
Volume ($m^3 \times 10^6$)	1.9	2.4	11.1	4.5	56.5
Mean depth (m)	2.9	3.1	7.4	4.1	11.9
Maximum depth (m)	0.5	6.6	17.2	9.5	27.5
pH	8.3	8.5	8.4	8.1	8.5
Alkalinity (mg $CaCO_3 \cdot L^{-1}$)	176	211	145	163	169
TP ($g \cdot L^{-1}$)	67	69	32	65	35

Note: Source: E.E. Prepas (unpublished data) and Mitchell and Prepas (1990).
[a]South basins only.

reduce mean summer total phosphorus (TP) and chlorophyll *a* concentrations by as much as 70 and 88%.

Macroinvertebrates are a key component of aquatic food webs; therefore, the potential impacts of lime application on macroinvertebrate communities are of concern, particularly to fisheries management programs. Although the impact of lime application on macroinvertebrate communities in soft-water lakes has been widely reported (e.g., Olem et al. 1991), the effects on benthic fauna in hard water has received little attention. A previous study reported the effects of lime application on littoral macroinvertebrates in a hard-water dugout (i.e., Miskimmin et al. 1995); however, their study design was inherently compromised and before–after treatment comparisons were absent. Predicting the effects of $Ca(OH)_2$ additions on macroinvertebrates from hardwater lakes is difficult because the effects of lime additions on the water column and sediment–water interface are poorly understood and experimental studies that could identify likely benthic responses are lacking. For instance, $Ca(OH)_2$ may negatively affect macroinvertebrates by physically restricting their movement when phosphate and phytoplankton cells coprecipitate with calcite (Zhang and Prepas 1996*b*) and form a precipitate layer at the sediment–water interface. Additionally, increased water column pH immediately following $Ca(OH)_2$ applications, as a result of the dissociation of hydroxide ions, could reduce survival of taxa sensitive to increased pH. In the longer term, lime applications could conceivably decrease food resources for benthic detritivores as reduced P concentrations result in declines in phytoplankton biomass.

In the present study, we quantified macroinvertebrate responses to $Ca(OH)_2$ treatment in two eutrophic hardwater lakes on the Boreal Plain. Community characteristics of littoral and shallow profundal macroinvertebrates in the two lakes were compared before and after a single $Ca(OH)_2$ addition. At the same time, macroinvertebrate data were collected from three reference lakes in the same region. In situ studies were then compared with laboratory experiments that examined three possible mechanisms by which $Ca(OH)_2$ could affect macroinvertebrate communities: $Ca(OH)_2$ dosage, pH, and the presence of precipitate at the sediment–water interface. By utilizing a combination of field observations and laboratory experiments, we were able to explore the potential for $Ca(OH)_2$ to affect macroinvertebrate communities. This study was part of a larger study on the impacts of single and multiple treatments of $Ca(OH)_2$ on water quality and biotic communities in eutrophic hardwater lakes.

Materials and methods

Field experiments

Five naturally eutrophic study lakes were selected in the Boreal Mixedwood Ecoregion of Alberta (Table 1). The untreated (designated reference) lakes (Jenkins, Crooked (south basin), and Baptiste (south basin)) are larger and deeper than the treated lakes (Lofty and North Halfmoon). Nonetheless, their chemical and biological characteristics (e.g., pH, ion and nutrient concentrations, invertebrate taxa and biomass) are within a comparable range (Table 1; Figs. 1 and 2). North Halfmoon and Lofty lakes were treated once with 225 and 295 g $Ca(OH)_2 \cdot m^{-2}$ (74 and 107 $mg \cdot L^{-1}$) in June 1991 and July 1992, respectively. For both lakes, the $Ca(OH)_2$ was added over 8–10 days. $Ca(OH)_2$ was applied as a slurry over the surface of North Halfmoon Lake via booms attached to the side of a barge. Direct treatment of nearshore areas where the water depth was <2 m was not possible with the barge; however, wind mixing ensured that the slurry eventually covered most of the lake. At Lofty Lake, a truck-mounted unit mixed $Ca(OH)_2$ with lake water and a boat distributed the slurry over the water surface with a series of booms and nozzle sprayers. The shore-based distribution system was developed for smaller waterbodies and allowed direct treatment of shoreline areas.

Macroinvertebrates were collected from the five study lakes at 3-week intervals between May and August from 1991 to 1993. In each lake, samples were taken along three transects at water depths of 1, 3, and 5 m. These sampling depths included benthic organisms from the littoral (1 and 3 m) and shallow profundal zones (5 m) of each lake. Two samples were collected with an Ekman grab sampler (15 × 15 × 23 cm) at each water depth for a total of 18 samples per sampling date per lake. In the field, samples were rinsed through a 500-μm sieve and preserved in 4% formalin. A modified subsampling procedure was used to reduce the time spent sorting samples (Sebastien et al. 1988). Each sample was subdivided into four equal subsamples by weight. We determined that macroinvertebrates were randomly distributed in the subsample by calculating the index of dispersion for five samples (Wrona et al. 1982). One subsample was sorted for macroinvertebrates. In the laboratory, macroinvertebrates were sorted into six taxonomic groups: Chironomidae, Chaoboridae, Amphipoda, Ceratopogonidae, Oligochaeta, and an "other" category representing numerically rare taxa. Mean densities were calculated for each taxonomic group at each water depth within a transect. The means for the nine

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Table 2. Equipment and procedures for analyzing 16 environmental variables in the five study lakes.

Parameter	Method
Temperature	Montedoro–Whitney model TC-5C resistance thermometer (1990–1991); Flett thermistor (1991–1993)
Light	Protomatic model 313 light meter (1990–1991); LiCor underwater quantum sensor (1991–1993)
TP and TDP	Modified (Prepas and Rigler 1982) potassium persulfate method (Menzel and Corwin 1965)
DO	Water samples fixed in the field and titrated within 24 h following Winkler DO method (Carpenter 1965)
pH	Metrohm E588 pH meter measured within 1 h of sample collection
Conductivity	Metrohm E587 conductometer measured within 1 h of sample collection
Total alkalinity	Potentiometric titration method completed within 24 h of sample collection (Environment Canada 1979)
NO_3^-, NO_2^-	Technicon autoanalyzer measured within 48 h of sample collection (Cd reduction method, Stainton et al. 1977)
NH_4^+	Technicon autoanalyzer measured within 48 h of sample collection (modified from the phenol–hypochlorite method, Solórzano 1969)
Cl^-, SO_4^{2-}	Ion chromatography with Dionex analyzer (APHA et al. 1989)
Ca^{2+}, Na^+, K^+, Mg^{2+}	Filtered (Whatman GF/C, 1.2 μm) before analysis on a Perkin-Elmer model 3030 atomic absorption spectrophotometer (APHA et al. 1989)

Note: Water samples were collected at the deepest site in each lake at 1- or 2-m intervals from surface to maximum lake depth.

sampling stations in each lake were then averaged to give mean densities per square metre per sampling date for each lake.

Dry (60°C, 24 h) weight biomass estimates were determined from preserved samples to the nearest 0.1 mg on a Mettler AT261 DeltaRange balance for total macroinvertebrates and individuals within each of the six taxonomic groups. Mean total biomass (grams per square metre) was estimated for the May–August periods of 1991–1993.

For each lake, water samples were collected and analyzed for 16 environmental variables every 2 weeks from May to August in 1991–1993 (Table 2). A vertical profile of whole-water samples was collected at the deepest site in each lake with an aluminum drop-sleeve water bottle sampler (1.5 L, 30 cm in height) at discrete 1- or 2-m intervals from the lake surface to maximum lake depth. Temperature and light absorbance (photosynthetically active radiation (PAR)) were measured at 1-m intervals from the surface of the lake to the bottom.

Repeated measures ANOVAs were used to compare differences in mean densities of individual taxonomic groups, total mean densities of macroinvertebrates, and total biomass estimates per sampling date between treated and reference lakes. For these analyses, the five lakes were blocked into two categories (treated or reference), and mean densities and total biomass estimates of macroinvertebrates were compared between the 15 sampling periods over the 3-year study at 1, 3, and 5 m. Similarly, repeated measures ANOVAs were used to test for differences in total and mean densities of individual taxa and total biomass estimates between treated and reference lakes at 1, 3, and 5 m during the sampling periods immediately before (pretreatment) and 21 days after $Ca(OH)_2$ application (posttreatment). For these analyses, the five lakes were blocked into two categories (treated or reference), and densities of macroinvertebrates by individual taxa and densities and biomass estimates of all taxa groups combined were compared up to 6 days before and 21 days after $Ca(OH)_2$ treatment. Pairwise comparisons were performed with Newman–Keuls tests. Differences ($P \leq 0.05$) in water chemistry immediately before and 21 days after $Ca(OH)_2$ treatment were examined with t tests. To satisfy normality and homogeneity of variance, original data were $\ln(x + 1)$ transformed. All statistical analyses were performed with SPSS (SPSS Inc. 1993).

The relationship between macroinvertebrate density and environmental variables was determined for each lake with canonical correspondence analysis (CCA) (CANOCO v. 3.12, ter Braak 1988, 1990). Macroinvertebrate abundance and environmental data were $\ln(x + 1)$ transformed prior to CCA to ensure that data were normally distributed, and detrended correspondence analysis (DCA) was performed on abundance data to determine the gradient length of the first ordination axis (CANOCO v. 3.12, ter Braak 1988, 1990). For each lake, CCA was determined to be the most appropriate approach to detect patterns in macroinvertebrate taxa groups and taxa group–environment relationships, since DCA indicated a wide gradient length (2–3 SD from the mean, ter Braak and Prentice 1988).

Sampling depth and the 16 environmental variables described in Table 2 were included in the initial CCA. Calcium concentrations in North Halfmoon Lake and total dissolved phosphorus (TDP) concentrations in Crooked Lake had high colinearity with other variables (i.e., variance inflation factors >20, ter Braak 1988). These variables were removed from further CCA in the appropriate lakes. A series of CCA tests were performed on each lake with the forward-selection option; at each step, added variables were tested for statistical significance with Monte Carlo permutations (99 unrestricted permutations, $P \leq 0.05$). Macroinvertebrate taxon points and the arrows of the environmental variables in an ordination biplot reflect the dominant patterns in community composition that can be best explained by environmental variables. The length of the arrow determines the influence of an environmental variable on the macroinvertebrate data (i.e., longer arrow means greater influence). Perpendicular lines drawn from an arrow to macroinvertebrate taxon points determine the relative positions of the taxa group distribution along that environmental axis. In each lake, the significance of the first canonical ordination axis was then tested with Monte Carlo tests (99 unrestricted permutations, $P \leq 0.05$).

Laboratory experiments

Three experiments were performed sequentially to identify possible mechanisms by which $Ca(OH)_2$ applications could alter macroinvertebrate abundance and biomass. During the summer of 1994 and 1995, we determined survivorship of *Chironomus* spp. and *Hyalella azteca* after 96-h exposure to (*i*) 0 (control), 100, 150, 200, or 300 g $Ca(OH)_2 \cdot m^{-2}$ (0, 594, 891, 1188, and 1781 $mg \cdot L^{-1}$), (*ii*) a pH of 8 (control), 9, 10, or 11 with or without a precipitate layer; and (*iii*) the presence or absence (control) of a physical layer (200 g diatomaceous earth (DE)$\cdot m^{-2}$) overlying the sediments at pH 8 and 10. We chose *Chironomus* spp. and *H. azteca* as test organisms because they are ubiquitous in Boreal Plain hardwater lakes and make use of subsurface and surface benthic habitat, respectively. Amounts of $Ca(OH)_2$ used reflected the strongest treatments to date in lakes and dugouts (300 $g \cdot m^{-2}$). The dosages applied to deeper lakes (per square metre) were applied to the laboratory microcosms that were only a few centimetres deep; there-

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fore, the microcosms received very concentrated treatments. Targets for pH were chosen to reflect those normally found in surface waters on the southern portion of the Boreal Plain (pH of 8 to low 9s) and extremes (pH of 10 to 11) that can be reached if enough chemical is added in a short period of time.

Cylindrical Plexiglas microcosms (volume = 2 L, bottom surface area = 95 cm^2) were filled with water and sieved sediments (≤500-μm particle size) at a 3:1 volume ratio. Surface water and bottom sediments were obtained from the deepest site (9.5 m) of Crooked Lake 72 h before each experiment. *Hyalella azteca* were collected from the littoral zone (1 m) of Crooked Lake, whereas *Chironomus* spp. were collected from the profundal zone (9.5 m), 24 h before each experiment. Microcosms were kept at 21°C under a 16 h light : 8 h dark cycle and gently aerated. Ten individuals of *Chironomus* spp. or *H. azteca* were introduced into the microcosms and allowed to acclimate for 24 h before treatment. The density of individuals per microcosm was similar to the mean densities of chironomids and amphipods in Crooked Lake during the summer of 1991 (800 ± 160 and 977 ± 209 individuals·m^{-2}, respectively). Each treatment was replicated 5 times and test organisms were not supplementally fed during the 96-h experimental period. At the end of the experiments, microcosm contents were passed through a 250-μm-mesh sieve to retrieve test organisms. Survivorship was determined by recording the number of live individuals. An organism was considered alive if it moved after being probed gently with forceps. Conductivity (μS·cm^{-1}) and DO concentrations (mg·L^{-1}) were measured at the sediment–water interface at 0 and 96 h for all experiments. DO concentrations were determined with micro-Winkler techniques (Burke 1962). In all experiments, DO concentrations remained above 3 mg·L^{-1}. DO concentrations increased slightly throughout the course of all three 96-h experiments but remained ≤6 mg·L^{-1}.

To determine the impact of $Ca(OH)_2$ dosage (including a precipitate layer) on macroinvertebrate survival, dosages of 0, 100, 150, 200, and 300 g $Ca(OH)_2$·m^{-2} (0, 594, 891, 1188, and 1781 mg·L^{-1}) were added to the experimental chambers. On a volumetric basis, the initial concentrations were 6–25 times higher in the microcosms than in the treated lakes; however, much of the $Ca(OH)_2$ added to the microcosms settled out within the first 12 h of the experiment. The areal dosages of $Ca(OH)_2$ used in the experiment were within the range of those applied to our treated lakes (i.e., 225 and 295 g·m^{-2}). We chose our experimental dosages to test what would happen in extreme hardwater conditions (i.e., high concentrations added in shallow water). One millilitre of lake water was mixed with 0.95, 1.43, 1.90, or 2.85 g of $Ca(OH)_2$ to form a slurry. The slurry was then added to the microcosms slowly and evenly across the water surface to form a homogeneous layer on the sediments and attain final dosages of 100, 150, 200, and 300 g $Ca(OH)_2$·m^{-2} in the chambers, respectively. Carbon dioxide was bubbled into the microcosms to control pH throughout the experiment.

After determining what dosage of $Ca(OH)_2$ could be applied without significant mortality to *Chironomus* spp. and *H. azteca*, subsequent experiments were conducted to identify what pH level was tolerable for *Chironomus* spp. and *H. azteca*. In these pH experiments, microcosms were treated with either concentrated $Ca(OH)_2$ or NaOH (1.0 N) to reach pH 8, 9, 10, or 11. The amount of chemical added was small (and undetermined) relative to the $Ca(OH)_2$ dosage experiments. Microcosms were checked for pH at 0, 2, 4, 8, 12, 24, 48, 72, and 96 h after treatment with a portable Beckman 10 pH meter (± 0.01 unit) and additional drops of NaOH or $Ca(OH)_2$ were occasionally added at the high-pH treatments to ensure that the pH remained at treatment level. The purpose of having NaOH and $Ca(OH)_2$ treatments at different pHs was to evaluate the role of pH with and without a precipitate layer, since NaOH does not form a precipitate. Test organisms were exposed to pH levels higher than those observed during lime applications to hardwater lakes (i.e., pH 11) to determine the pH range that resulted in mortality. We chose pH 8 as control in the laboratory experiments because open-water pH of the study lakes ranged between 8.1 and 8.6. We could then compare the effect of pH changes in the laboratory with that in the natural habitat of the test organisms.

The final set of laboratory experiments was designed to test the extent to which the presence of a precipitate layer affected the survival of *Chironomus* spp. and *H. azteca*. In these experiments, DE (200 g·m^{-2}) was added as a slurry across the water surface in the microcosms and pH was maintained at 8 or 10 with NaOH (1.0 N). The result was a visual precipitate similar to that obtained at the maximum dosage of $Ca(OH)_2$. DE closely resembles the precipitate layer formed by calcium phosphate (i.e., both produce a fine precipitate), yet it is nearly chemically inert. The pH values (8 and 10) were chosen because they bound the range of minimum and maximum values recorded during whole-lake application of $Ca(OH)_2$ to hardwater lakes in Alberta (Prepas et al. 1990; Babin et al. 1994). Further, they bound the range of values observed in the euphotic zone of eutrophic hardwater lakes in this region during the daytime.

For laboratory bioassays, one- or two-factor ANOVAs were used to test the effects of (*i*) different dosages of $Ca(OH)_2$, (*ii*) different water column pHs maintained with NaOH or $Ca(OH)_2$, and (*iii*) the presence or absence of a precipitate layer (DE) overlying the sediment at pH 8 and 10 on *H. azteca* and *Chironomus* spp. survival. Differences between treatment means were tested for with Newman–Keuls post hoc tests. To satisfy normality and homogeneity of variance, original data were arcsine, square root transformed.

Results

Field experiments

Dominance among macroinvertebrate groups varied among study years for each lake (Fig. 1). In North Halfmoon Lake, Chironomidae, Amphipoda, and Chaoboridae were the dominant taxa during the treatment year (1991), comprising 85% of all macroinvertebrates. A year after treatment, Chironomidae and Oligochaeta were numerically dominant, comprising 86% of total macroinvertebrate density. Mean abundance of Chironomidae was 7-fold higher in 1992 than in 1991 (24 766 and 3599 individuals·m^{-2}, respectively), and Oligochaeta mean abundance was 30 times higher in 1992 compared with 1991 (29 270 and 969 individuals·m^{-2}, respectively). However, 2 years after treatment, Chironomidae, Amphipoda, and Chaoboridae were the most numerically dominant groups as in pretreatment conditions. In Lofty Lake, Amphipoda always dominated except on one sampling date in June 1991 (Fig. 1). Chironomidae, Amphipoda, and Oligochaeta dominated reference lakes throughout the study period, comprising 79–85% of mean total macroinvertebrate densities (Fig. 1). As in the treated lakes, dominant taxa in the reference lakes varied substantially between years.

Total densities of macroinvertebrates were generally 1–2 times greater in the reference relative to treated lakes, both immediately before and 21 days after $Ca(OH)_2$ application ($F = 9.85$, $P < 0.01$, df = 1, 45) and over the 3-year study ($F = 2.29$, $P = 0.01$, df = 14, 126) (Fig. 1). Within a given year, densities of individual groups were generally highest during July and August in both the treated and reference lakes (Fig. 1). However, temporal patterns differed between treated and reference lakes, largely due to oligochaete densities ($F = 2.22$, $P = 0.01$, df = 14, 126). In North Halfmoon Lake, densities of oligochaetes increased threefold 1 year af-

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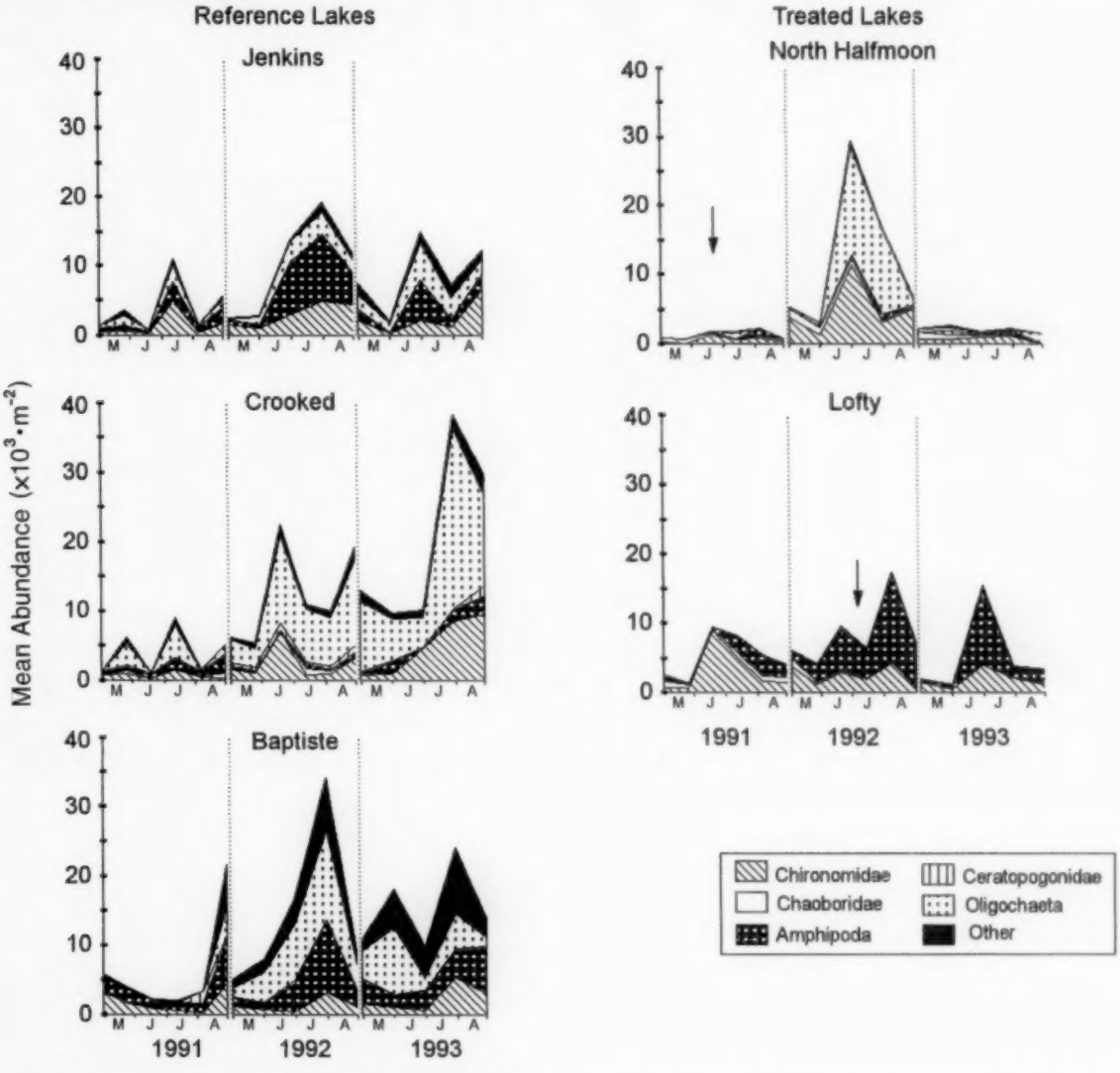
Fig. 1. Mean abundance of numerically dominant macroinvertebrate taxa in the reference and treated lakes from 1991 to 1993. Arrows indicate the date of lime treatment.



ter treatment compared with the treatment year (42 and 12% of total macroinvertebrate density, respectively) but returned to pretreatment conditions 2 years after treatment (Fig. 1). In contrast, oligochaete densities in Lofty Lake remained very low and unchanged from 1991 to 1993. In the reference lakes, oligochaete densities increased progressively over the study period.

Except for Lofty Lake in 1991 and North Halfmoon Lake in 1992, total biomass estimates of macroinvertebrates (all taxa) varied within a given year but were not detectably different among years within each lake (Fig. 2). Total biomass often peaked in early May and late July, likely because of the presence of large reproducing adults and recruitment of young, respectively. Total biomass estimates did not differ between the treated and reference lakes either immediately before or after $Ca(OH)_2$ application ($F = 0.03$, $P = 0.87$, df = 1, 18) or over the 3-year study period ($F = 0.65$, $P = 0.82$, df = 14, 126) (Fig. 2).

Densities of major macroinvertebrate taxa and total biomass estimates at any given depth were similar among treated and reference lakes before and 21 days after the application of 225 and 295 g $Ca(OH)_2 \cdot m^{-2}$ (74 and 107 $mg \cdot L^{-1}$) to North Halfmoon and Lofty lakes, respectively (i.e., no detectable difference among densities at 1, 3, and 5 m; $P > 0.2$). In all five lakes, chaoborid densities were generally highest at 5 m compared with 1 and 3 m depths ($F = 12.78$, $P < 0.01$, df = 2, 45), but this pattern was reversed for amphipods ($F = 4.72$, $P = 0.01$, df = 2, 45). In the treated lakes, amphipod and chironomid densities generally were not detectably different immediately before and 21 days after treatment (Table 3). Although densities of oligochaetes and macroinvertebrates in the category "other" increased 21 days after

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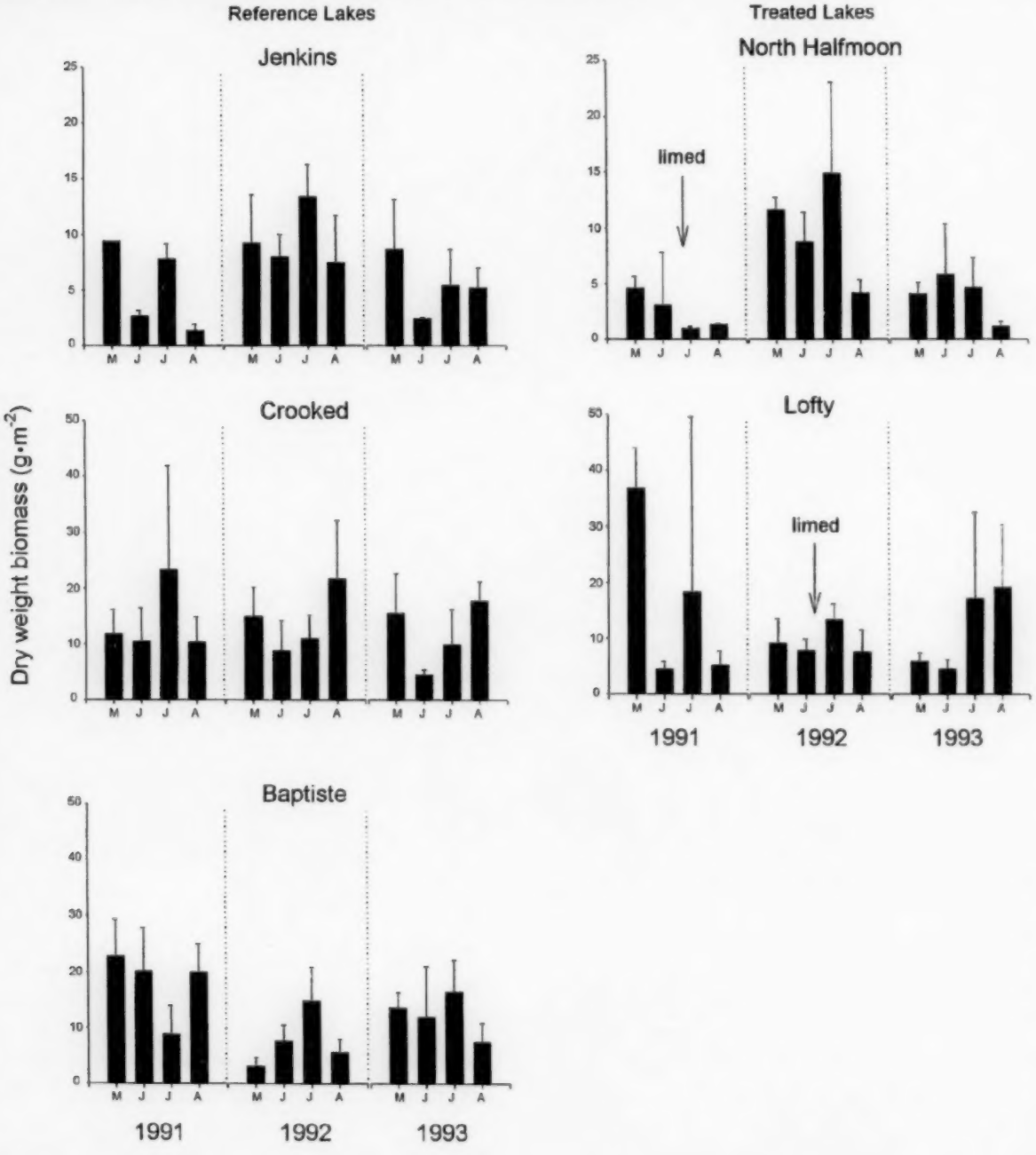
Fig. 2. Mean dry weight biomass of total benthic macroinvertebrates in the reference and treated lakes from 1991 to 1993. Note the variation in the scale of the vertical axes. Arrows indicate the date of lime treatment.



treatment in North Halfmoon Lake, similar increases were also observed in the reference lakes (Table 3). Moreover, densities of all major taxa in Lofty and North Halfmoon lakes 42 days after treatment were not detectably different from pretreatment levels. In the reference lakes, amphipod, chironomid, oligochaete, and "other" macroinvertebrates did not differ detectably before and 21 days after treatment of Lofty Lake (Table 3).

For all five lakes, CCA revealed strong correlations between macroinvertebrate density and environmental variables, which explained 95–99% of the variance in macroinvertebrate density (Table 4). However, the eigenvalues of

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Table 3. Mean densities (± SE) of major benthic macroinvertebrate taxa in North Halfmoon, Lofty, and reference lakes immediately before and 21 days after $Ca(OH)_2$ treatment in 1991 and 1992.

Year	Lake	Treatment	Total	Chir	Chao	Amph	Cera	Olig	Other
1991	North Halfmoon	Before	1 821	1363	313	123	15	0	7
			(620)	(620)	(96)	(56)	(10)	(0)	(5)
		After	1 769	644	89	0	22	960	54
			(751)	(225)	(35)	(0)	(9)	(545)	(19)
	Reference	Before	1 328	495	30	505	111	131	56
			(255)	(175)	(20)	(97)	(41)	(39)	(21)
		After	7 926	2178	111	1729	154	3047	707
			(2976)	(1069)	(81)	(415)	(44)	(1765)	(286)
1992	Lofty	Before	9 627	2928	227	5848	10	333	281
			(2728)	(861)	(80)	(2075)	(8)	(201)	(59)
		After	6 332	1931	155	3887	2	141	215
			(2028)	(631)	(40)	(1528)	(2)	(122)	(68)
	Reference	Before	21 509	3864	11	5456	820	9297	2060
			(4855)	(1621)	(8)	(3247)	(347)	(2338)	(748)
		After	25 070	3379	320	7933	219	9219	3999
			(5601)	(819)	(219)	(3288)	(118)	(2500)	(1400)

Note: Means include samples from all three depths. Chir, Chironomidae; Chao, Chaoboridae; Amph, Amphipoda; Cera, Ceratopogonidae; Olig, Oligochaeta; Other, all other taxonomic groups.

the first two axes of CCA were low (Fig. 3), cumulatively explaining only 10–34% of total variation in taxa–environment relations (Table 4). These results suggest that much of the taxa variance (i.e., variance in density) could be related to the environmental variables measured, since Monte Carlo permutations of the first canonical axes were significant ($P = 0.01$). However, the same variables did not predict density for all lakes. The forward-selection criteria determined that pH, water temperature, sampling depth, DO, TP, and Mg concentrations explained the weighted mean taxa densities almost as well as the original 17 variables for the lakes (sampling depth and the 16 variables listed in Table 2). Hence, only the smaller subset is presented in Table 4. With the exception of Baptiste Lake, sampling depth and pH contributed to either axis 1 or 2 in the CCA biplot of all lakes. Sampling depth and pH were correlated with axis 1 for Lofty, North Halfmoon, and Crooked lakes, whereas Mg concentration was correlated with axis 2 ($r = 0.2$, 0.4, and 0.4, respectively). In contrast, Mg concentration ($r = -0.4$) and sampling depth ($r = 0.3$) were correlated with axes 1 and 2, respectively, for Baptiste Lake. For Jenkins Lake, the length of the depth and pH arrows and their proximity to axes 1 and 2, respectively, indicate that these variables were most strongly correlated with the axes. Water temperature and DO concentrations were important in explaining the variation in biplot distribution of macroinvertebrates in Lofty and Crooked lakes, while TP concentrations were a significant contributor to explaining macroinvertebrate distribution in Lofty, North Halfmoon, and Jenkins lakes. The biplot distribution of macroinvertebrates in the treated lakes was correlated with water column pH, sampling depth, water temperature, TP, and Mg concentrations.

The CCA biplots (Fig. 3) also indicated that in all the lakes, chaoborid densities were higher in deeper regions, whereas amphipods were more abundant in the shallower areas. In addition, most taxa groups appeared to be restricted to intermediate pH values of 8–9. However, oligochaete densities were associated with higher Mg concentrations in the treated than in the reference lakes (Fig. 3). In both North Halfmoon and the reference lakes, high densities of oligochaetes and "other" macroinvertebrates 21 days after $Ca(OH)_2$ treatment (Table 3) coincided with an increase in water temperature from 15 to 19°C (Table 5, $P < 0.01$). In both treated lakes, densities of amphipods and chironomids did not change detectably 21 days after $Ca(OH)_2$ treatment, despite an average pH increase of 0.7 ± 0.1 unit (Table 5, $P < 0.01$); however, water column pH was always below 10. Distinct groups of pre- versus post-treatment sample points were not present in the CCA biplots for North Halfmoon and Lofty lakes (Fig. 3), suggesting that macroinvertebrate distribution patterns did not change as a result of $Ca(OH)_2$ application.

Laboratory experiments

Survivorship of *Chironomus* spp. over 96 h did not differ between microcosms that received anywhere from 0 to 300 g $Ca(OH)_2 \cdot m^{-2}$ ($F = 0.33$, $P = 0.86$, df = 4, 20) (Fig. 4A). In contrast, more *H. azteca* died at 300 g$\cdot m^{-2}$ compared with dosages of 0–200 g $Ca(OH)_2 \cdot m^{-2}$ ($F = 7.14$, $P = 0.01$, df = 4, 20) (Fig. 4B). However, it is unclear whether the mortality of *H. azteca* at high pH was the result of dosage or pH, since pH in the 300 g$\cdot m^{-2}$ experimental chambers spiked (range 8.8–10.6) 24 h into the experiment (Fig. 5).

In the pH experiments, more than 75% of all chironomids survived for 96 h at pH 8, 9, 10, and 11, regardless of whether NaOH or $Ca(OH)_2$ was used to maintain the desired pH ($F = 0.88$, $P = 0.46$, df = 3, 32) (Fig. 4C). On the other hand, *H. azteca* mortality was 50 and 100% at pH 10 and 11, respectively, for both NaOH and $Ca(OH)_2$ treatments ($F = 79.00$, $P < 0.01$, df = 3, 32) (Fig. 4D). Post hoc tests indicated no detectable difference in mortality of *H. azteca* between pH 8 and 9 but differences between all other pairwise

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Table 4. CCA of littoral and profundal macrobenthos in five eutrophic hardwater lakes.

Lake	Taxa data, first two axes	Taxa–environment relationship, first two axes	Environmental variable	Canonical coefficient		Correlation coefficient	
				Axis 1	Axis 2	Axis 1	Axis 2
Lofty	34.2	97.2	pH	–0.5	–0.5	–0.6	–0.2
			Depth	0.6	–0.2	0.6	0.1
			Mg	–0.5	0.9	–0.4	0.2
			Temperature	0.1	–0.7	–0.2	–0.2
			TP	–0.2	0.2	0.1	0.1
North Halfmoon	24.1	95.3	pH	0.4	0.4	0.4	–0.1
			Depth	–0.7	0.7	–0.6	0.2
			Mg	0.5	0.9	0.3	0.4
			TP	0.2	–0.5	–0.1	–0.1
Jenkins	9.6	99.3	pH	–0.7	0.7	–0.2	0.3
			Depth	–0.6	–0.4	–0.2	–0.2
			TP	0.6	0.3	0.2	0.2
Crooked	24.6	98.8	pH	0.1	0.2	0.2	0.1
			Depth	–0.9	0.4	–0.7	0.1
			Mg	0.1	0.9	0.1	0.4
			DO	0.1	0.4	0.4	0.2
Baptiste	13.6	97.1	pH	–0.5	–0.2	–0.4	–0.2
			Depth	0.1	0.7	0.2	0.3
			Mg	–0.7	0.6	0.4	0.2

Note: The first two axes of the ordinations explained 10 and 34% of the overall variation in benthic macroinvertebrate density data. The ordinations identified three to five environmental variables (pH, sampling depth, temperature, Mg concentration, and TP concentration) important in explaining 95–99% of the variation in the taxa data. Canonical and correlation coefficients of environmental variables to the first two axes of the ordinations are shown.

combinations. Given that the amount of chemical added to increase pH in the water of the microcosms was very little, relative to that added in the dosage experiments, we conclude that the effect of precipitate is minimal in these pH experiments. Thus, within the time frame of these experiments, *H. azteca*, but not *Chironomus* spp., was sensitive to pH ≥10.

The presence of DE marginally affected the survival of *Chironomus* spp. ($F = 5.07$, $P = 0.05$, df = 1, 8) (Fig. 4E) at both pH 8 and 10. In contrast, DE had no detectable effect on the survival of *H. azteca* ($F = 0.05$, $P = 0.82$, df = 1, 16) (Fig. 4F). Hence, it appears that survival of *Chironomus* spp., but not *H. azteca*, may be depressed by the presence of a precipitate. As demonstrated in previous experiments, however, *H. azteca* was sensitive to pH shifts as high as 10.

Discussion

Macroinvertebrate community structure is naturally variable in the eutrophic hardwater lakes sampled. Within this context, applications of 225 and 295 g $Ca(OH)_2 \cdot m^{-2}$ (74 and 104 $mg \cdot L^{-1}$) did not detectably impact total biomass, density, and dominance of major macroinvertebrate taxa either in the short (21 days after treatment) or long term (1–2 years after treatment). This result is consistent with that of Sarkar and Konar (1984) who found that chironomid densities did not change after 90 days of exposure to 225 g agricultural $lime \cdot m^{-2}$ (1350 $mg \cdot L^{-1}$) in hardwater ponds, despite sediment pH increasing from 7.0 to 8.5. In contrast, Miskimmin et al. (1995) inferred that pH increases of 3 or more units (from 8 to 12) were responsible for higher chironomid and lower amphipod densities in the treated compared with reference side of a hardwater pond in Alberta 63 days after application of 350 g $Ca(OH)_2 \cdot m^{-2}$ (250 $mg \cdot L^{-1}$). However, their interpretations are fundamentally limited because invertebrate densities were not measured before $Ca(OH)_2$ treatment and pH was not monitored during the experiment. Studies on $Ca(OH)_2$ or $CaCO_3$ applications to softwater lakes have reported increased densities and biomass of macroinvertebrates following lime applications. For example, Raddum et al. (1984) reported increased densities of mayflies, oligochaetes, and chironomids 1–2 years after 1.1 kg $CaCO_3 \cdot m^{-2}$ was applied to Lake S. Boksjø, Sweden. Similarly, total biomass of benthic fauna increased 2 years after $CaCO_3$ treatment of acidic lakes in Sweden (Appelberg 1990). Increasing pH from 5 to 9 in softwater lakes with $CaCO_3$ additions may increase densities by altering filtering rates and internal ionic balance and may also allow colonization by acid-sensitive taxa such as crustaceans and ephemerids (Stephenson and Mackie 1986). Thus, reduced acid-stressed conditions after lime treatment in softwater lakes, compared with highly buffered hardwater lakes, may explain observed differences in benthic responses between these water bodies.

Chironomids and amphipods are known to move through the water column to emerge and feed, while chaoborids exhibit vertical diurnal migration to avoid predation and feed on zooplankton (Post and Cucin 1984). We expected that in-

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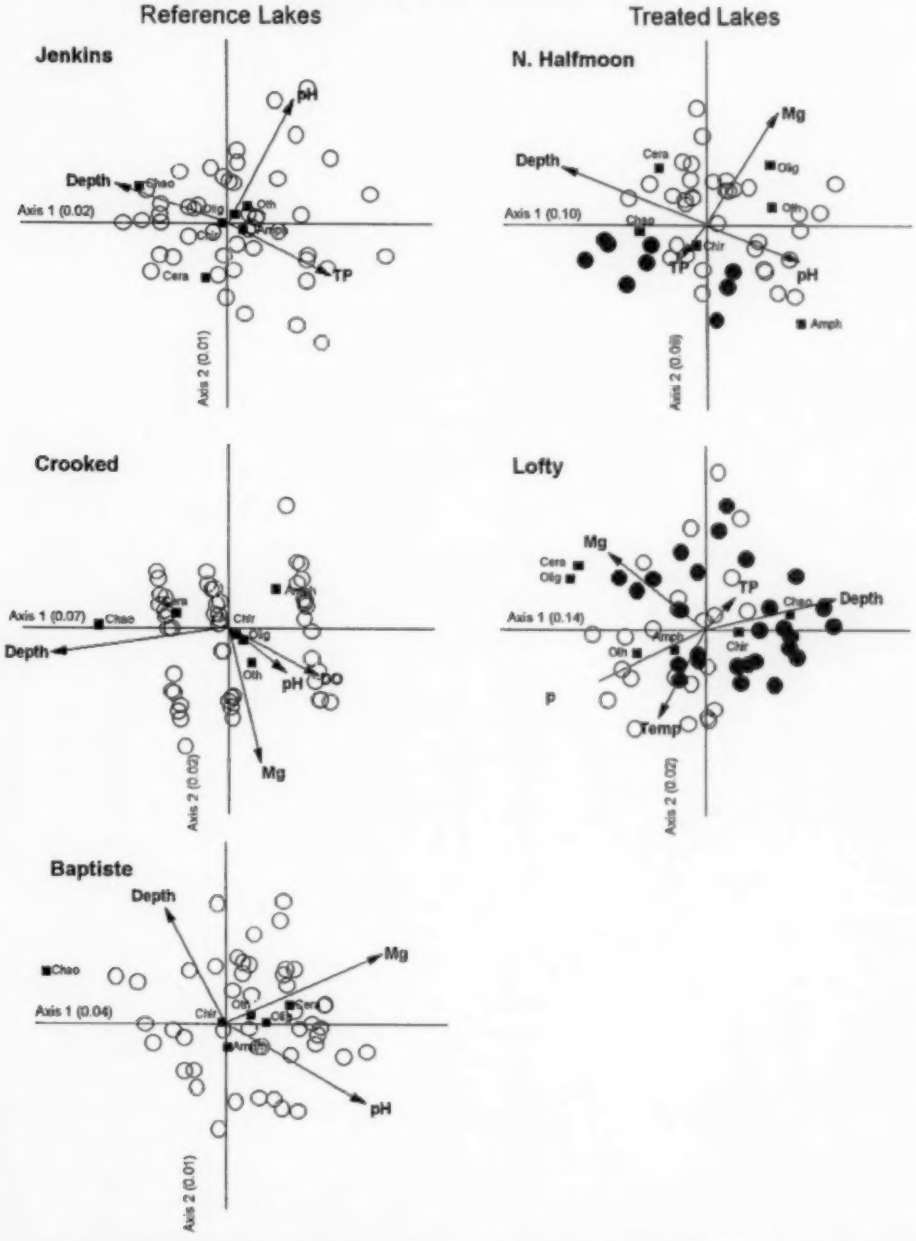
Fig 3. Ordinations of taxa by CCA for the five study lakes. Eigenvalues for axes 1 and 2 are shown in parentheses following the axes labels. Environmental variables are represented by arrows, taxa by squares, sample scores for reference lakes and treated lakes (preliming) by open circles, and postliming treatment scores by solid circles. Chir, Chironomidae; Chao, Chaoboridae; Amph, Amphipoda; Cera, Ceratopogonidae; Olig, Oligochaeta; Oth, all other taxonomic groups



creased water turbidity following $Ca(OH)_2$ application would temporarily disrupt the movement patterns of these taxa and, consequently, influence their densities at different depths. Densities of these groups were not detectably different among depths in the treated lakes both before and after $Ca(OH)_2$ application. Therefore, if turbidity was a factor after $Ca(OH)_2$ treatment, it did not markedly alter benthic macroinvertebrate densities.

Our 4-day laboratory experiments demonstrated that dosages of ≤ 200 g $Ca(OH)_2 \cdot m^{-2}$ and a pH maintained below 10 were not harmful to chironomids or amphipods. However, dosages of 300 g $Ca(OH)_2 \cdot m^{-2}$ and (or) pH ≥ 10 decreased amphipod, but not chironomid, survivorship. Perhaps organisms occupying surface benthic habitat such as *H. azteca* may be more sensitive to pH fluctuations in the open water than those living in the bottom sediments. While extrapola-

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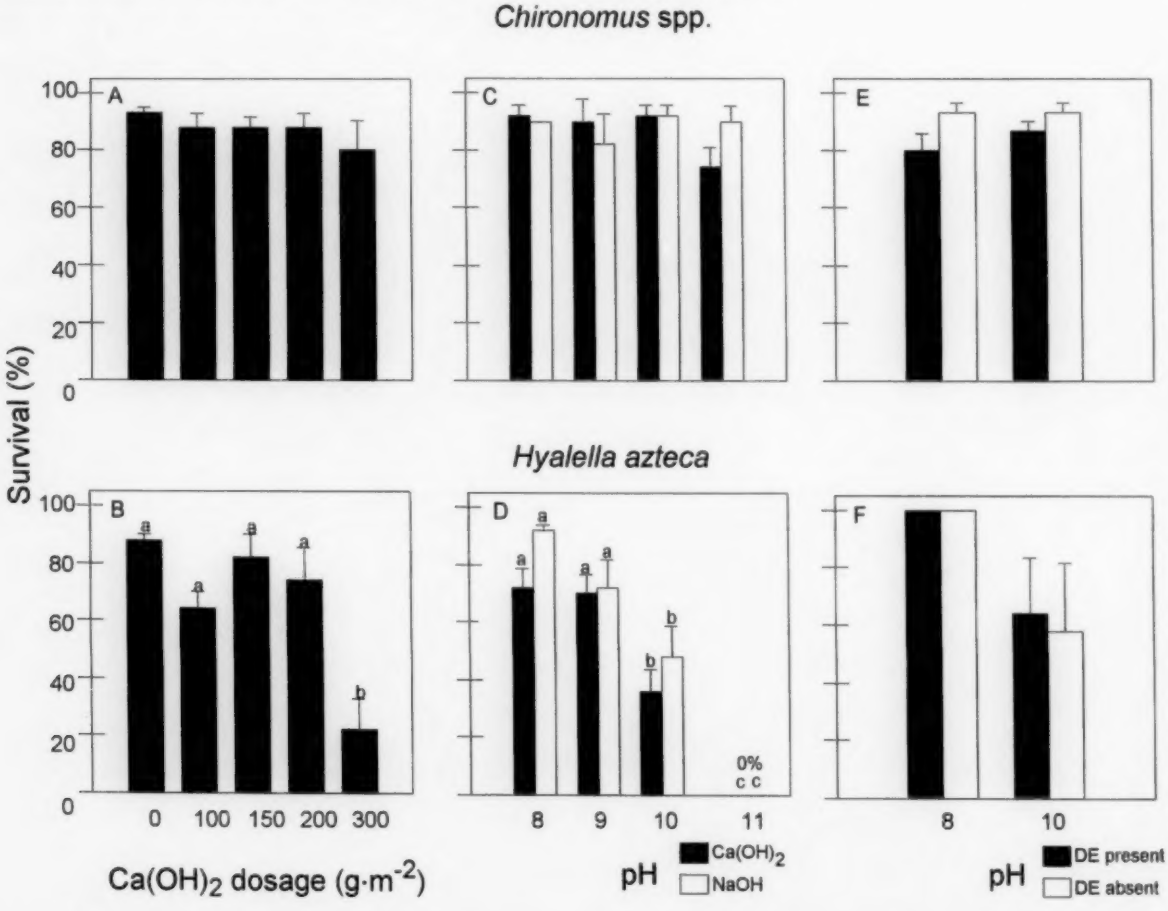
Table 5. Mean (±SE) pH, water temperature, TP concentration, and Mg concentration in North Halfmoon, Lofty, and three reference lakes immediately before and 21 days after $Ca(OH)_2$ treatment in 1991 and 1992.

Year	Lake	Treatment	pH	Temperature (°C)	TP ($\mu g \cdot L^{-1}$)	Mg ($mg \cdot L^{-1}$)
1991	North Halfmoon	Before	8.0 (0.0)**	15.4 (2.6)**	61.7 (2.6) ns	24.9 (0.1)*
		After	8.7 (0.0)	19.2 (0.5)	67.3 (5.8)	24.1 (0.3)
	Reference	Before	8.1 (0.0) ns	15.0 (0.1)**	32.6 (1.4) ns	12.7 (0.2)**
		After	8.1 (0.1)	19.2 (0.2)	37.2 (2.9)	13.2 (0.2)
1992	Lofty	Before	8.5 (0.2)**	18.5 (0.4) ns	70.1 (7.5)*	32.6 (0.1)**
		After	9.3 (0.0)	19.1 (0.3)	50.2 (4.5)	30.0 (0.1)
	Reference	Before	8.6 (0.1) ns	19.4 (0.2) ns	45.0 (2.7) ns	16.0 (0.1)**
		After	8.5 (0.1)	19.3 (0.1)	53.6 (6.0)	17.1 (0.2)

Note: $*P < 0.05$; $**P < 0.01$; ns, $P > 0.05$.

Fig 4. Survivorship (± SE) of *Chirnonmus* spp. and *H. azteca* (A and B) at five dosages of $Ca(OH)_2$, (C and D) at four different pH conditions maintained with NaOH or $Ca(OH)_2$, and (E and F) in the presence and absence of DE at two different pH conditions. Significant differences between treatments are indicated with different lowercase letters; no letter indicates no detectable difference. The difference in survival of *Chironomous* spp. in the presence or absence of DE was marginal ($P = 0.05$) at both pH levels. Untransformed data were used to calculate SE. The absence of error bars indicates SE = 0.



tion of laboratory microcosms to field situations should be made cautiously (Carpenter 1996), our laboratory experiments suggest that marked declines in macroinvertebrate densities would not be expected in field experiments such as ours where water column pH was <10. However, our results are based on macroinvertebrates from littoral and shallow

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profundal sites and may not apply to organisms from deep profundal sites.

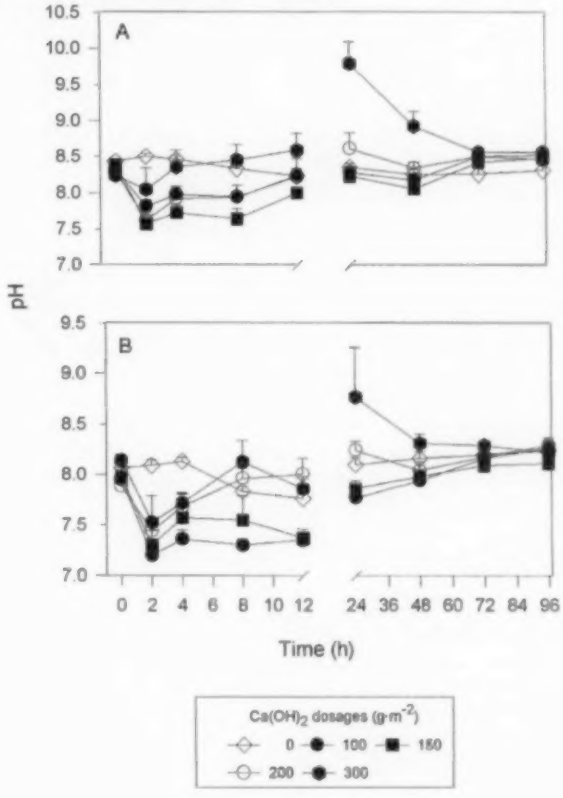
Although amphipod and chironomid densities in situ did not change detectably after $Ca(OH)_2$ treatment, oligochaete densities increased temporarily in North Halfmoon Lake and the reference lakes. This density increase may have resulted from an enhanced development rate caused by a natural summer increase in water temperature, as suggested in our CCA analyses.

Benthic macroinvertebrate communities are highly variable both within and among years. For example, in temperate hardwater lakes and ponds, strong monthly and yearly fluctuations in macroinvertebrate density and biomass are common (Dinsmore and Prepas 1997). In our study, mean population densities of oligochaetes in Baptiste Lake varied within a given year from 1254 to 12 883 individuals·m^{-2} in early May and late July, respectively, and between years from 123 to 12 883 individuals·m^{-2} in July of 1991 and 1992, respectively. This natural variability reduced our ability to detect an effect of lime addition on benthic communities; however, we attempted to minimize this problem by sampling macroinvertebrate communities at 3-week intervals throughout the growing season for three years (1991–1993). Moreover, our analyses included comparisons of benthic responses in treated lakes as well as untreated reference lakes. Given this approach, we believe that the absence of a detectable effect of lime application on macroinvertebrates likely reflects the absence of biologically meaningful impacts. Lastly, the absence of a detected treatment effect in the field experiments is consistent with results predicted from our laboratory experiments.

Alternatively, the lack of detectable change in macroinvertebrate abundance following lime treatment of hardwater lakes may be indicative of the ability of macroinvertebrates to recover rapidly from environmental perturbations. Lotic benthic macroinvertebrates recover quickly from a variety of perturbations (see Mackay 1992). While few studies have documented the ability of lentic macroinvertebrates to recover from disturbances, the fact that many taxa present in our study lakes have short life histories, ranging from weeks to months, suggests that they also should be able to recolonize lake areas even if substantial mortality had occurred.

We expect that the spatial and temporal variability in benthic macroinvertebrate communities among our five study lakes over the study period was likely a response to a suite of environmental factors and life history patterns that were unaffected by lime additions. Despite high temporal variation within lakes, other investigators have attempted to develop predictive models to explain variation in macroinvertebrate abundance between lakes (e.g., Rasmussen and Kalff 1987). In our study, pH, sampling depth, water temperature, DO, TP, and Mg concentrations were important predictors of macroinvertebrate density, but those predictors varied among lakes. This resulted in ordinations that explained only 10–34% of total taxa variance, suggesting that their utility as predictive models may be low. It is possible that the interaction of chemical (e.g., Mg and Ca concentrations) or biological (e.g., predation, competition) variables may account for some of the unexplained variation in littoral and shallow profundal benthic community structure. It is

Fig. 5. Mean (± SE) pH during $Ca(OH)_2$ experiments for (A) *H. azteca* and (B) *Chironomus* spp.



also possible that environmental variables estimated from the water column may be only loosely related to conditions at the sediment–water interface. Clearly, our ability to predict the effects of $Ca(OH)_2$ treatment on the macroinvertebrate communities of eutrophic hardwater lakes will improve as the basic environmental factors that govern their natural life histories are better understood.

In conclusion, where maintenance of benthic macroinvertebrate community structure is a concern, dosages of $Ca(OH)_2$ that maintain pH <10 are not likely to cause a detectable change in the community. With the exception of small or shallow water bodies, pH is unlikely to increase above 10 in hardwater lakes, as, based on current technology, it often takes several days to apply $Ca(OH)_2$ to most water bodies. By considering the environmental factors that influence benthic macroinvertebrate communities in eutrophic hard waters, lake managers can develop criteria for lake remediation that prevent unwanted impacts on benthic organisms.

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References

APHA et al. 1989. Standard methods for the examination of water and wastewater. 17th ed. American Public Health Association, Washington, D.C.

Appelberg, M. 1990. Population regulation of the crayfish *Astacus astacus* L. after liming an oligotrophic, low-alkaline forest lake. Limnologica, **20**: 319–327.

Babin, J., Prepas, E.E., Murphy, T.P., Serediak, M., Curtis, P.J., Zhang, Y., and Chambers, P.A. 1994. Impact of lime on sediment phosphorus release in hardwater lakes: the case of hypereutrophic Halfmoon Lake, Alberta. Lake Reservoir Manage. **8**: 131–142.

Brinkhurst, R.O. 1974. The benthos of lakes. Macmillan Press Ltd., London, England.

Burke, J.D. 1962. Determination of oxygen in water using a 10-mL syringe. J. Elisha Mitchell Sci. Soc. **78**: 145–147.

Carpenter, J.H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol. Oceanogr. **10**: 141–143.

Carpenter, S.R. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology, **77**: 677–680.

Dinsmore, W.P., and Prepas, E.E. 1997. Impact of hypolimnetic oxygenation on profundal macroinvertebrates in a eutrophic lake in central Alberta. I. Changes in macroinvertebrate abundance and diversity. Can. J. Fish. Aquat. Sci. **54**: 2157–2169.

Environment Canada. 1979. Analytical methods manual. Inland Water Directorate, Water Quality Branch, Ottawa, Ont.

Jónasson, P.M. 1978. Zoobenthos of lakes. Verh. Int. Ver. Limnol. **20**: 13–37.

Kotak, B.G., Kenefick, S.L., Fritz, D.L., Rousseaux, C.G., Prepas, E.E., and Hrudey, S.E. 1993. Occurrence and toxicological evaluation of cyanobacterial toxins in Alberta lakes. Water Res. **27**: 495–506.

Mackay, R.J. 1992. Colonization of lotic macroinvertebrates: a review of processes and patterns. Can. J. Fish. Aquat. Sci. **49**: 617–628.

Menzel, D.W., and Corwin, N. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. Limnol. Oceanogr. **10**: 280–282.

Miskimmin, B.M., Donahue, W.F., and Watson, D. 1995. Invertebrate community response to experimental lime ($Ca(OH)_2$) treatment of an eutrophic pond. Aquat. Sci. **57**: 20–30.

Mitchell, P.A, and Prepas, E.E. 1990. Atlas of Alberta Lakes. University of Alberta Press. Edmonton, Alta.

Olem, H., Schreiber, R.K., Brocksen, R.W., and Porcella, D.B. 1991. International lake and watershed liming practices. The Terrene Institute, Inc., Washington, D.C.

Post, J.R., and Cucin, D. 1984. Changes in the benthic community of a small Precambrian lake following the introduction of yellow perch, *Perca flavescens*. Can. J. Fish. Aquat. Sci. **41**: 1496–1501.

Prepas, E.E., and Rigler, F.H. 1982. Improvements in quantifying the phosphorus concentration in lake water. Can. J. Fish. Aquat. Sci. **39**: 822–829.

Prepas, E.E., Murphy, T.P., Crosby, J.M., Walty, D.T., Lim, J.T., Babin, J., and Chambers, P.A. 1990. Reduction of phosphorus and chlorophyll *a* concentrations following $CaCO_3$ and $Ca(OH)_2$ additions to hypereutrophic Figure Eight Lake, Alberta. Environ. Sci. Technol. **24**: 1252–1258.

Raddum, G.G., Hagenlund, G., and Halvorsen, G.A. 1984. Effects of lime treatment on the benthos of Lake Søndre Boksjø. Inst. Freshwater Res. Drottningholm Rep. **61**: 167–176.

Rasmussen, J.B., and Kalff, J. 1987. Empirical models for zoobenthic biomass in lakes. Can. J. Fish. Aquat. Sci. **44**: 990–1001.

Sarkar, S.K., and Konar, S.K. 1984. Effects of agricultural lime on fish and aquatic ecosystem. Environ. Ecol. **2**: 171–175.

Sebastien, R.J., Rosenberg, D.M., and Wiens, A.P. 1988. A method for subsampling unsorted benthic macroinvertebrates by weight. Hydrobiologia, **157**: 69–75.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenol–hypochlorite method. Limnol. Oceanogr. **14**: 799–801.

SPSS Inc. 1993. SPSS for Windows: advanced statistics, release 6.0. SPSS Inc., Evanston, Ill.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Publ. No. 25.

Stephenson, M., and Mackie, G.L. 1986. Lake acidification as a limiting factor in the distribution of the freshwater amphipod *Hyalella azteca*. Can. J. Fish. Aquat. Sci. **43**: 288–292.

ter Braak, C.J.F. 1988. CANOCO — a FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal components analysis and redundancy analysis. Version 2.1. Agriculture Mathematics Group, 6700 AC Wageningen, The Netherlands.

ter Braak, C.J.F. 1990. Update notes: CANOCO, version 3.12. Agricultural Mathematics Group, 6700 AC Wageningen, The Netherlands.

ter Braak, C.J.F., and Prentice, I.C. 1988. A theory of gradient analysis. Adv. Ecol. Res. **18**: 271–317.

Wrona, F.J., Culp, J.M., and Davies, R.W. 1982. Macroinvertebrate subsampling: a simplified apparatus and approach. Can. J. Fish. Aquat. Sci. **39**: 1051–1054.

Zhang, Y., and Prepas, E.E. 1996*a*. Regulation of the dominance of planktonic diatoms and cyanobacteria in four eutrophic hardwater lakes by nutrients, water column stability, and temperature. Can. J. Fish. Aquat. Sci. **53**: 621–633.

Zhang, Y., and Prepas, E.E. 1996*b*. Short-term effects of $Ca(OH)_2$ additions on phytoplankton biomass: a comparison of laboratory and in situ experiments. Water Res. **30**: 1285–1294.

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Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies

Sharon Z. Herzka and G. Joan Holt

Abstract: The stable isotope composition of larval fish tissues may serve as a chemical tracer of recent settlement due to food web differences among planktonic and demersal habitats. We present the background for the utilization of $\delta^{13}C$ and $\delta^{15}N$ to trace settlement of red drum (*Sciaenops ocellatus*), an estuarine-dependent species. The effect of ontogeny and temperature on the relative contribution of growth and metabolic turnover to changes in isotopic composition was examined by simulating dietary shifts in the laboratory. Fractionation was examined as a function of size and the effect of food deprivation was evaluated. Published growth rates were used to estimate the time period within which the isotopic composition of a new food source should be reflected in larval tissues. In response to dietary shifts, larvae exhibited quick changes in $\delta^{13}C$ and $\delta^{15}N$ in a pattern closely resembling predictions based on growth alone. Fractionation values were about +1‰ for $\delta^{13}C$ and +1.6‰ for $\delta^{15}N$. There was no effect of 4 days of food deprivation on $\delta^{13}C$ and $\delta^{15}N$. Given the fast growth rates reported for newly settled red drum, their isotopic composition should exhibit a shift within 1–2 days and stabilize about 10 days following settlement.

Résumé : La composition en isotopes stables des tissus des larves de poisson peut servir de traceur chimique de l'implantation récente dans la zone benthique étant donné les écarts trophiques existant entre les habitats planctonique et benthique. Nous donnons le contexte de l'utilisation des rapports $\delta^{13}C$ et $\delta^{15}N$ pour suivre l'implantation du tambour (*Sciaenops ocellatus*), une espèce estuarienne. Les effets de l'ontogénie et de la température sur les apports relatifs de la croissance et de la transformation métabolique sur la composition isotopique ont été examinés en provoquant des changements du régime alimentaire en laboratoire. Le fractionnement a été étudié en fonction de la taille et de l'absence de nourriture. Les taux de croissance publiés ont été appliqués à l'estimation du temps nécessaire pour que la composition isotopique d'une nouvelle source de nourriture se reflète dans les tissus des larves. En réponse au changement de leur régime alimentaire, les larves ont présenté des variations rapides des $\delta^{13}C$ et $\delta^{15}N$ dont l'allure correspondait de près aux prévisions fondées sur la croissance seule. Les valeurs du fractionnement étaient environ de +1‰ pour le $\delta^{13}C$ et de +1,6‰ pour le $\delta^{15}N$. Une privation de nourriture de 4 jours n'a pas eu d'effet sur ces rapports. Étant donné les taux de croissance élevés signalés pour les tambours récemment implantés sur les fonds, leur composition isotopique devrait présenter un décalage de 1 à 2 jours et se stabiliser environ 10 jours plus tard.

[Traduit par la Rédaction]

Introduction

Recruitment variability is often attributed to processes acting upon the early life of marine fish species. Settlement to demersal nursery habitat has been identified as an important component of recruitment and is influenced by a host of physical and biological processes acting on the pre- and post-settlement stages (Boehlert and Mundy 1988). Consequently, the relationship between planktonic larval supply and settlement to nursery habitat must be examined on a variety of carefully chosen spatial and temporal scales. The logistic difficulties and high cost of such encompassing studies have resulted in trade-offs between the simultaneous study of large-scale spatial and fine-scale temporal patterns of early life settlement dynamics (Doherty and Williams 1988).

Studies addressing settlement dynamics have relied on a variety of techniques, including length–frequency analysis, visual sensing, and the identification of dietary shifts or specific developmental stages associated with settlement (e.g., Leis 1991; Grover et al. 1998). Otolith settlement marks have been used to estimate time since settlement on an individual basis (Victor 1991). In addition, there is recent evidence that otolith microchemistry may prove useful for tracing movement of larval and juvenile fishes (Thorrold et al. 1997). The utilization of these techniques has allowed for considerable progress in the study of the early life dynamics of fishes.

Unfortunately, these techniques are not suitable for some marine fish species, particularly when seeking to address questions pertaining to the fine-scale temporal dynamics of settlement. The resolution of length–frequency distributions is limited by the sampling interval (Leis 1991), and it can be difficult to distinguish among individuals that have settled at different times. In addition, visual techniques are not suit-

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able for turbid areas or cryptic larvae, and not all species exhibit an otolith settlement mark or pronounced developmental stage that can be associated with the settlement transition. Hence, the identification of additional chemical or morphological characters that could be used to estimate the time since settlement of fish larvae on an individual basis would be beneficial.

Animal tissues reflect the isotopic composition of their food sources in a predictable manner (Peterson and Fry 1987). Variations in food web structure and available food sources among habitats can result in differences in the isotopic composition of resident organisms. Stable isotope ratios have been used to identify the change in habitat of estuarine larval and juvenile fishes (Deegan et al. 1990), trace migration patterns of juvenile shrimp and fish (Fry 1983; Hesslein et al. 1991), and distinguish among populations of adult fishes (Roelke and Cifuentes 1997). Since rapidly growing animals quickly reflect the isotopic composition of a new diet (Fry and Arnold 1982), the isotopic composition of larval fish tissues may change after settlement as a result of a dietary shift. Hence, stable isotope ratios may serve as tracers of recent arrival to nursery habitat.

The utilization of stable isotope ratios as tracers of settlement requires estimates of the rate of change in the isotopic composition of larval fish tissues following a dietary shift. For growing animals, the isotopic composition of newly added biomass will reflect that of the current food source (Fry and Arnold 1982). Simultaneously, metabolic turnover results in the breakdown and replacement of existing body tissues (Tieszen et al. 1983). As a result, the rate of isotopic change is a function of both growth and metabolic turnover.

We present estimates of the rate of change in $\delta^{13}C$ and $\delta^{15}N$ of red drum (*Sciaenops ocellatus*) larvae subjected to dietary shifts under laboratory conditions. Red drum settle from coastal waters to estuarine nursery habitat early in their life cycle (Holt et al. 1983; Rooker et al. 1998). Although abundance pulses of recent recruits have been observed, the fine-scale temporal resolution of settlement patterns is limited. Red drum settle at a range of sizes (4–8 mm standard length (SL); Rooker et al. 1998), and the use of specific developmental stages to trace settlement is not suitable. In addition, since red drum larvae lack a clearly defined otolith settled mark (S.A. Holt, Marine Science Institute, University of Texas at Austin, 750 Channelview Drive, Port Aransas, TX 78373, U.S.A., and J.R. Rooker, Texas A&M University, 5007 Avenue U, Galveston, TX 77551, U.S.A., personal communication), it is currently not possible to estimate the time since settlement for individuals. Lastly, movement of recently settled larvae within the nursery habitat cannot be differentiated from settlement.

The data presented provide the foundation for using stable isotope ratios to examine the temporal dynamics of settlement of red drum and other marine fish species that exhibit a distinct habitat and (or) dietary change as a result of settlement. Our objectives were to (*i*) determine the influence of ontogenetic state and temperature on the contribution of growth and metabolic turnover to the rate of $\delta^{13}C$ and $\delta^{15}N$ change following a dietary shift, (*ii*) evaluate size-dependent fractionation, short-term food deprivation, and the size of the carbon and nitrogen pool throughout ontogeny, and (*iii*) derive a realistic estimate of the time within which the isotopic composition of a recently settled red drum larva should change based on published growth rates for postsettlement red drum.

Materials and methods

Red drum larval culture

Red drum eggs were obtained from adults spawning under controlled temperature and photoperiod at the Marine Science Institute, University of Texas at Austin (Arnold 1988). Eggs hatch after 24 h and larvae commence feeding on day 3 posthatch after yolk-sac reserves are almost depleted (Holt et al. 1981*a*). Larvae were reared at initial densities of 2000 fish in 150-L conical tanks (Holt 1993) and kept on a 12 h light : 12 h dark photoperiod in a temperature-controlled laboratory. All experiments were completed at salinities of 30–35‰.

Dietary shift experiments

The change in $\delta^{13}C$ and $\delta^{15}N$ of red drum larvae in response to a diet of differing isotopic composition was evaluated for first-feeding (2.6 mm SL) and settlement-size (4–8 mm SL) fish. The experiment at first feeding was completed at 28°C, within the temperature for optimum growth of early red drum larvae (Holt et al. 1981*b*). Isotopic shifts for settlement-size red drum were evaluated at 24 and 28°C, within the 19–31°C encountered by populations in nursery habitat (Rooker and Holt 1997). The isotopic compositions of food sources and larvae used in the dietary switch experiments described below are listed in Table 1.

The initial $\delta^{13}C$ and $\delta^{15}N$ of larvae prior to the initiation of feeding (day 3) was determined by collecting 15 larvae from each of two experimental tanks. Larvae were subsequently fed rotifers (*Brachionus plicatilis*) grown on an algal monoculture (*Nannochloropsis oculata*) (Holt 1992). Collections were completed for the next 7 days. Due to their small size (mean SL = 2.6 mm on day 3 to 4.7 mm on day 10), larvae collected from any one tank were later pooled for stable isotope ratio analysis (5–15 individuals, $n = 2$ samples per day). Following collection, larvae were held in filtered seawater for 2–4 h to allow for gut clearance (G.J. Holt, unpublished data). Red drum were then anesthetized with a minute quantity of MS 222 and SL measurements were obtained using a Wilde stereomicroscope and Summa Sketch digitizing table. Larvae were stored in clean glass autosampler tubes and dried at 60°C for 24–48 h.

Isotopic shifts for settlement-size red drum were examined by feeding larvae rotifers grown on *N. oculata* from the initiation of feeding until the target size (about 6–7 mm SL) was achieved; the median size was 6.4 mm SL for the 28°C experiment (day 13) and 6.6 mm SL for the 24°C experiment (day 23). Larvae were switched to freshly hatched *Artemia fransiscana* (San Francisco Bay Brand, Supreme 99, Salt Lake Brine Shrimp, Newark, Calif.) nauplii fed daily. Twenty larvae were collected prior to the dietary switch and every 1 or 2 days for the following 10 days and processed as described previously. Due to the large size variability of cultured red drum and the need to examine $\delta^{13}C$ and $\delta^{15}N$ shifts as a function of accurate growth measurements, the four larvae most closely approximating the median SL for each sampling date were selected and stable isotope ratio analyses were completed on those individual larvae.

Fractionation and food deprivation

Fractionation can be defined as the difference in isotopic ratios between an animal and its food source (Peterson and Fry 1987). We hypothesized that fractionation exhibited by fish larvae could be size dependent due to the potential effect of ontogenetic development on assimilatory capacity and metabolism. Hence, we examined fractionation as a function of size.

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Table 1. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of food sources and red drum larvae used during dietary switch and fractionation experiments and DWs of the larvae immediately prior to the dietary shift ($W_{initial}$).

	$W_{initial}$ (mg)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n
Dietary switch at first feeding (28°C)				
Rotifer culture		–23.7±1.3	–2.3±1.5	3
Red drum larvae (initial)	0.024±0.0004	–18.2±0.0	12.0±0.3	2[a]
Red drum larvae (final)		–23.9	0.9	—
Dietary switch at settlement size (28°C)				
Rotifer culture		–23.7±1.3	–2.3±1.5	3
Artemia nauplii		–22.1±0.1	10.7±0.2	2
Red drum larvae (initial)	0.74±0.12	–22.2±0.2	1.3±0.2	4[b]
Red drum larvae (final)		–20.5	12.2	—
Dietary switch at settlement size (24°C)				
Rotifer culture		–26.2±0.2	–3.8±1.2	3
Artemia nauplii		–22.1±0.1	10.7±0.2	2
Red drum larvae (initial)	0.89±0.84	–24.3±0.3	0.4±0.7	4[b]
Red drum larvae (final)		–20.9	14.1	—
Fractionation				
Rotifer culture		–23.7±1.3	–2.3±1.5	2
Artificial diet		–22.7±0.2	6.8±0.2	2

Note: Stable isotope ratios of the larvae following equilibration on the final food source (δ_f) represent asymptotic values derived through nonlinear curve fitting.

[a]Stable isotope ratio isotopic analysis completed on pooled samples of larvae (15).

[b]Stable isotope ratio isotopic analysis completed on individual larvae.

Because red drum cannot be raised on artificial diets alone and the variability in isotopic ratios of the rotifer culture (Table 1) was within the range of values typically observed as fractionation factors, red drum were weaned from rotifers to an artificial diet (Fry Feed Kyowa, Kyowa Hakko Kogyo Co., Japan) by day 8 posthatch (Holt 1993). A single batch of the diet was ground into different sizes (<250 to 500–700 μm) and provided to the larvae based on length. Larvae were fed throughout the day with automatic feeders. Ten individuals from each of three culture tanks were collected on days 11, 14, 17, and 20 posthatch (n = 30 per day) and processed as described for the dietary switch experiments above. Later, a total of 42 larvae spanning the full size range collected (4.6–15.2 mm SL) were selected for stable isotope analysis, which was completed on individuals. Fractionation was calculated as

$$(1) \quad \text{Fractionation factor } (\Delta) = (\delta_{animal} - \delta_{diet})$$

and examined as a function of SL using regression analysis.

The effect of short-term food deprivation on $\delta^{13}C$ and $\delta^{15}N$ of settlement-size red drum (mean size 6.7 mm SL) was evaluated by placing larvae reared on rotifers in beakers of filtered seawater. The isotopic composition of the larvae was determined for two or three individuals collected on days 0, 1, 2, and 4. The experiment was completed at 24°C. No mortality was observed during this time period.

Stable isotope analysis

Whole fish were finely ground and mixed thoroughly and a 400- to 800-μg sample used for isotopic determinations. Analysis of nine replicate subsamples of the same fish yielded a mean difference of 0.2 and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Rotifer cultures and *Artemia* nauplii were filtered onto precombusted GFA filters and dried at 60°C for 48 h. Ground fish or food samples were placed in tin boats; isotopic ratios and percent carbon and nitrogen were determined from a single sample using a Finnigan MAT DeltaPlus continuous-flow stable isotope mass spectrometer attached to a Carlo Erba elemental analyzer at the Marine Science Institute, University of Texas at Austin. Accuracy of isotopic measurements was verified by interspersing samples of a secondary standard (powdered chitin of marine origin, Sigma Aldrich Co., U.S.A., No. C-8908). Precision of chitin samples was usually 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Isotopic values are reported relative to PeeDee belemnite and atmospheric nitrogen using the standard equation

$$(2) \quad \delta_{sample}\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R represents the ratio of the heavy to light isotope.

Larvae collected during fractionation and dietary switch experiments were weighed to the nearest ±1 μg. The relationship between SL and dry weight (DW) was determined using the power function

$$(3) \quad W = a(SL)^b$$

where W represents DW and a and b are constants derived through nonlinear curve fitting using Sigma Plot software (Jandel Corporation). The percent carbon and nitrogen in red drum larvae was assessed as a function of SL and DW using regression analysis.

Contribution of growth and metabolic turnover to isotopic change

The $\delta^{13}C$ and $\delta^{15}N$ of red drum were expressed as a function of biomass gain relative to the DW of the larvae prior to the dietary switch, W_R:

$$(4) \quad W_R = \frac{W_t}{W_i}$$

where W_t is the DW of an individual larva at time t and W_i is the mean DW of the larvae selected for determination of initial $\delta^{13}C$ and $\delta^{15}N$.

Fry and Arnold's (1982) turnover equation was used to curve fit the data for each dietary switch experiment:

$$(5) \quad \delta_{Predicted} = \delta_f + (\delta_i - \delta_f)(W_R)^c$$

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where δ_i and δ_f are the initial and final isotopic values of the larvae, respectively. The value of c, the exponent of metabolic decay, is indicative of the relative contribution of growth and metabolic turnover to isotopic change (Fry and Arnold 1982). At $c = -1$, metabolic turnover is absent or not detectable and the equation resolves into a simple dilution model (i.e., mass balance) in which $\delta_{Predicted}$ is a function growth alone. For values of c less than -1, both growth and metabolic turnover contribute to the isotopic shift. The value of δ_i was calculated as the average value of individual fishes prior to the dietary switch and constrained during the curve-fitting procedure. The δ_f and c estimates were derived through nonlinear curve fitting. A Student's t test was utilized to test for significant differences in c for a given δ and treatment (initial size or temperature) and to determine if curve-fitted values of c differed significantly from -1. To estimate the percentage of variability explained by growth alone, linear regression analysis was completed on the measured δ values versus those predicted using the simple dilution model.

The contribution of the carbon or nitrogen present immediately prior to the dietary shift to the total carbon or nitrogen pool at a given relative biomass increase (W_R) can be estimated as

$$(6) \quad \%\text{ of total at } W_R = \frac{\delta_{Predicted} - \delta_f}{\delta_f - \delta_i} \times 100$$

where $\delta_{Predicted}$ is calculated using the values of c, δ_i, and δ_f derived from eq. 5. For example, using the simple dilution model ($c = -1$), a doubling in biomass ($W_R = 2$) corresponds to 1:1 dilution of the initial carbon by the addition of new carbon. Isotopic shifts expressed in this manner are independent of the absolute values of δ_i and δ_f and can be applied to any combination of initial and final $\delta^{13}C$ and $\delta^{15}N$.

Rate of isotopic shift under natural conditions

Growth rates observed under laboratory rearing conditions may not reflect those of natural populations. Hence, we utilized our data on the relative contribution of growth and metabolic turnover to isotopic change in conjunction with published growth rates to estimate the time that a planktonic signature will persist after a newly settled larva experiences a dietary shift. Rooker and Holt (1997) reported growth rates for postsettlement red drum collected in seagrass habitat in Aransas Bay, Tex. Using otolith increment counts to estimate size at age, they fitted an exponential model to their data:

$$(7) \quad SL = ae^{gt}$$

where SL is in millimetres, a is the estimated length at hatch (millimetres), g is the instantaneous growth coefficient (per day), and t is age (days). To estimate the daily biomass increase exhibited by red drum following settlement, the exponential growth model was integrated to yield

$$(8) \quad \frac{dSL}{dt} = gSL.$$

Integration of the length–weight relationship (eq. 3) and substitution into the integrated exponential growth model (eq. 8) results in

$$(9) \quad \frac{dW_t}{dt} = bgW_t$$

where b is the exponent of the length–weight relationship. The DW of a newly settled red drum was then calculated as a function of time after settlement:

$$(10) \quad W_R = \frac{W_t}{W_i} \text{ where } W_t = bgW_{(t-1)} + W_{(t-1)}.$$

The estimated relative biomass increase (W_R) exhibited by a larva on a given day after settlement and the exponent of metabolic decay (c) were subsequently used to estimate the contribution of the carbon or nitrogen present immediately prior to the dietary shift to the total pool as a function of growth:

$$(11) \quad \%\text{ of total at } W_R = W_R^c \times 100.$$

Because Rooker and Holt (1997) did not find a simple relationship between growth rate and ambient temperature for the six cohorts of postsettlement red drum that they examined, we evaluated the effect of growth rate variability on the rate of isotopic change by modeling its effect. We used the mean ($0.048 \cdot day^{-1}$), minimum ($0.041 \cdot day^{-1}$), and maximum ($0.058 \cdot day^{-1}$) instantaneous growth coefficients reported by Rooker and Holt (1997). For simplicity, a settlement size of 7 mm SL was chosen. Equations 10 and 11 are a function of the instantaneous growth coefficient and exponent of metabolic decay utilized; hence, the model is insensitive to the absolute settlement size used (data not shown).

Results

Dietary shift, fractionation, and food deprivation experiments

The shift in $\delta^{13}C$ and $\delta^{15}N$ of larval tissues toward δ_f was easily detectable following a doubling in biomass (Figs. 1 and 2), which corresponded to a 1- or 2-day period. Isotopic values equilibrated on the final food source after a roughly sixfold biomass increase. In most cases, the pattern in $\delta^{13}C$ and $\delta^{15}N$ resembled that predicted using a simple dilution model. However, the change in carbon isotopic composition exhibited by settlement-size larvae reared at 28°C appeared to occur slightly faster than that predicted using the simple dilution model, suggesting the possible contribution of metabolic turnover to isotopic change.

The $\delta^{13}C$ fractionation exhibited by red drum larvae relative to the artificial diet of constant isotopic composition was about +1‰ (Fig. 3*a*). Regression analysis indicated a significant relationship between $\delta^{13}C$ and SL ($p = 0.003$). However, the low proportion of the variability explained by the analysis ($r^2 = 0.20$) and small range in predicted $\delta^{13}C$ values based on model results (+0.8 and +1.2‰ for a 4- and 15-mm SL fish, respectively) suggest that a fractionation factor of +1‰ is representative for carbon. Due to a 9‰ difference in $\delta^{15}N$ between the live prey and the artificial diet onto which red drum were weaned (see Table 1), the isotopic composition of larvae of 4–8 mm SL (collected 11 and 14 days posthatch) had not completed turnover (Fig. 3*b*). These data were not included in the regression analysis. A fractionation of +1.6‰ was found for the size range of 9–15 mm SL (days 17 and 19), and there was no relationship between $\delta^{15}N$ and size ($p = 0.81$).

The $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) before the initiation of food deprivation were –24.0 ± 0.3 and 0.4 ± 0.1‰, respectively. Regression analysis of $\delta^{13}C$ and $\delta^{15}N$ as a function of time indicated that there was no significant relationship between isotopic composition and food deprivation period (Fig. 4). Hence, the $\delta^{13}C$ and $\delta^{15}N$ of settlement-size larvae were not sensitive to food deprivation within the time interval considered.

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Fig. 1. $\delta^{13}C$ of red drum larvae switched to a food source of differing isotopic composition at first-feeding and upon reaching settlement size. The relative biomass increase represents the DW of a larva at the time of collection normalized to the mean DW of larvae prior to the dietary switch. The dietary switch (W_R = 1) is indicated by an arrow. Solid lines represent model results derived from curve fitting Fry and Arnold's (1982) equation to the data to assess the relative contribution of growth and metabolic turnover to isotopic change. Coefficients of determination refer to curve fit results. The dotted line represents isotopic changes predicted using a simple dilution (growth only) model.

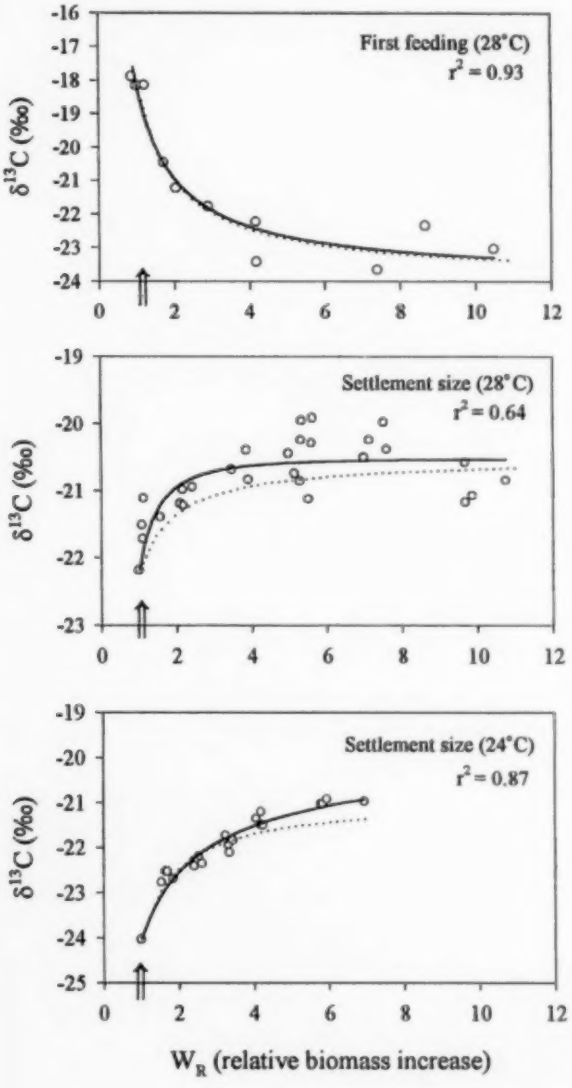
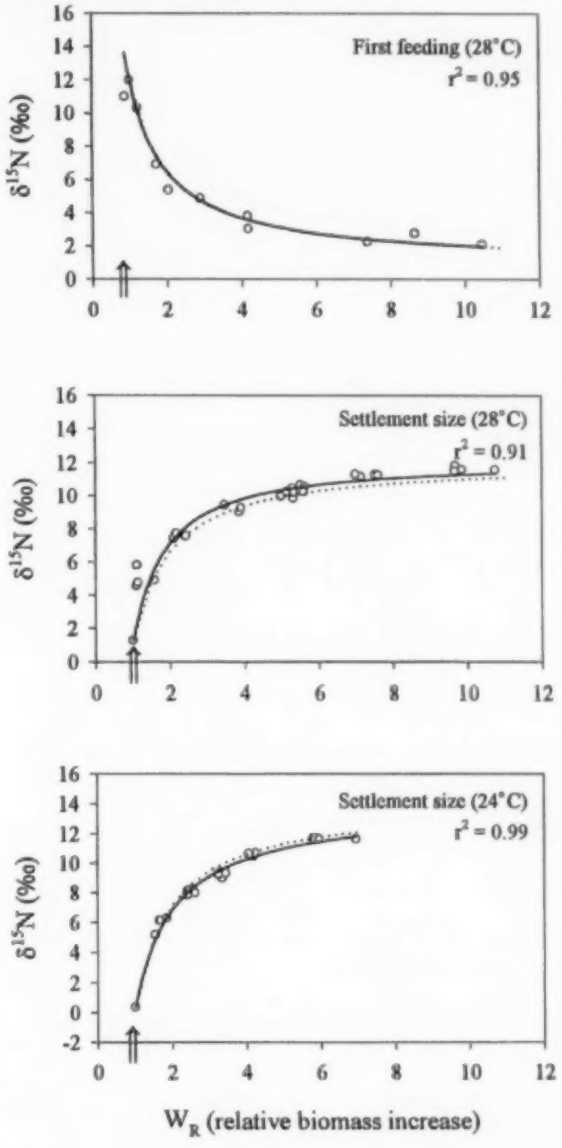


Fig. 2. $\delta^{15}N$ of red drum larvae switched to a food source of differing isotopic composition at first-feeding and upon reaching settlement size. See legend to Fig. 1 for details.



Percent carbon, percent nitrogen, and the SL–DW relationship

The percent carbon and nitrogen of red drum was about 40–45 and 10%, respectively. Regression analysis indicated that percent carbon increased significantly as a function of SL and DW ($p < 0.05$), while no significant relationships were observed for percent nitrogen ($p > 0.05$) (Fig. 5). Because of the low proportion of the variance explained by the regressions ($r^2 < 0.1$ in all cases) and the narrow range in percent carbon values observed for different sizes, no attempt was made to correct for the size of the carbon or nitrogen pool in modeling rates of isotopic change, which are therefore expressed as a function of DW.

The SL (millimetres) – DW (milligrams) relationship developed by curve fitting a power function to the data yielded

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Fig. 3. Linear regression and 95% confidence intervals of (*a*) $\delta^{13}C$ and (*b*) $\delta^{15}N$ fractionation factors calculated for individual red drum larvae fed an artificial diet of constant isotopic composition. Fractionation factors were calculated as $\delta_{animal} - \delta_{diet}$. The broken line represents no fractionation. Solid circles denote data included in regressions; open circles represent data that were not included in regressions because larvae had not equilibrated on the artificial diet.

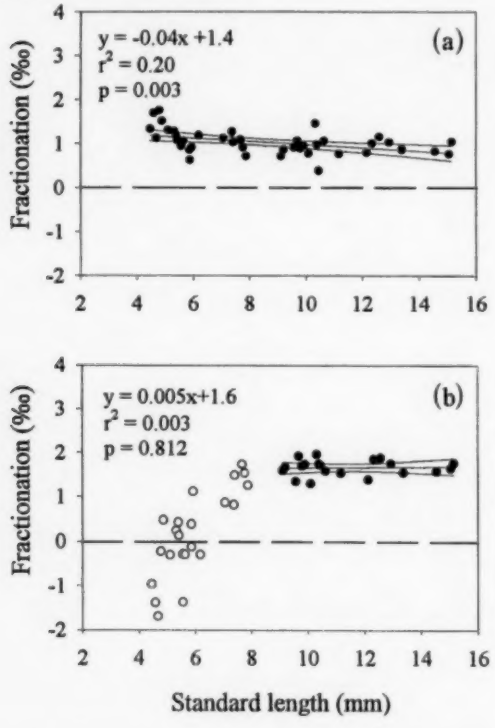
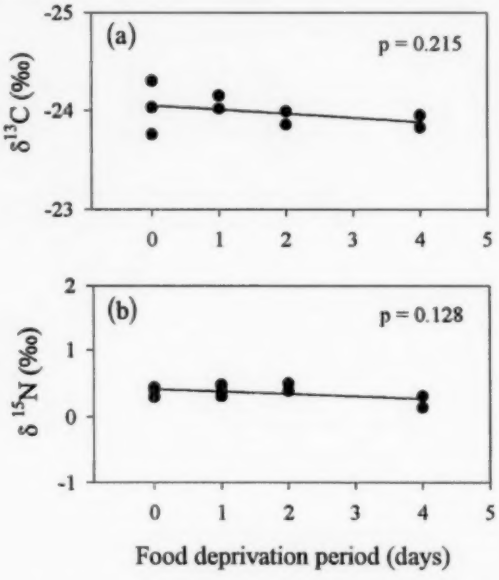


Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ of individual settlement-size red drum larvae subjected to a 4-day food deprivation period. Day 0 represents the isotopic composition of larvae prior to food deprivation. The solid lines and *p* values represent linear regression results.



the following relationship: $W = 0.0019SL^{3.3}$ ($r^2 = 0.98$, $n = 340$). The relationship was used to estimate the rate of isotopic change for natural populations as described in the Materials and methods section.

Contribution of growth and metabolic turnover to the rate of isotopic change

Curve-fitted values for the exponent of metabolic decay (*c*) approximated –1 for both ontogenetic states and rearing conditions, with the exception of $\delta^{13}C$ turnover for settlement-size larvae reared at 28°C, for which the value of *c* was about –2 (Table 2). However, statistical comparison of *c* values from (*i*) first-feeding and settlement-size larvae reared at 28°C and (*ii*) settlement-size red drum reared at 24 and 28°C yielded no significant differences between parameter estimates ($p > 0.05$). In addition, none of the parameter results were found to be significantly different from –1 (Table 2). Regression analysis of measured versus δ values predicted using the simple dilution model indicated that in most cases, growth explained >90% of the observed variability, although only 56% of the variability observed for settlement-size red drum reared at 28°C was attributable to growth (Table 2).

Statistical analysis of the exponent of metabolic decay values indicated that isotopic shifts can be adequately described using a simple dilution (growth only) model. Hence, the percent initial carbon or nitrogen comprising the total pool during growth was calculated using $c = -1$ and expressed as a function of relative biomass increase (W_R) (Fig. 6). As expected, the initial addition of biomass following a dietary shift should lead to rapid changes in the isotopic composition of larval tissues (Fig. 6). After a sixfold increase in biomass ($W_R = 6$), the initial carbon or nitrogen would account for only 20% of the total.

Turnover rates under natural conditions

An exponent of metabolic decay (*c*) of –1 was used to model the rate of dilution of initial carbon or nitrogen following a theoretical dietary shift occurring as a result of settlement to nursery habitat. Model results indicate that the addition of new carbon or nitrogen should occur quickly following a dietary shift; as a result, the $\delta^{13}C$ and $\delta^{15}N$ of newly settled red drum should change rapidly (Fig. 7). One day after settlement, only 75% of the existing carbon or nitrogen would correspond to that deposited prior to the settlement event. After 10 days, only about 20% of the initial carbon or nitrogen would comprise the initial pool at settlement. The variability in growth rates reported by Rooker and Holt (1997) resulted in about a 10% difference in contribution of carbon or nitrogen present at the time of settlement to the total pools as a function of time (Fig. 7).

Discussion

The assumptions underlying the interpretation of isotopic data have often been unsubstantiated, resulting in ambiguous conclusions (Gannes et al. 1997). To utilize stable isotope ratios to trace recent settlement of larval fishes, two assump-

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Fig. 5. Linear regression results of percent carbon and nitrogen of red drum larvae as a function of SL and DW (n = 340). Values were derived concomitant to stable isotope ratio analysis of larvae collected in fractionation and dietary switch experiments.

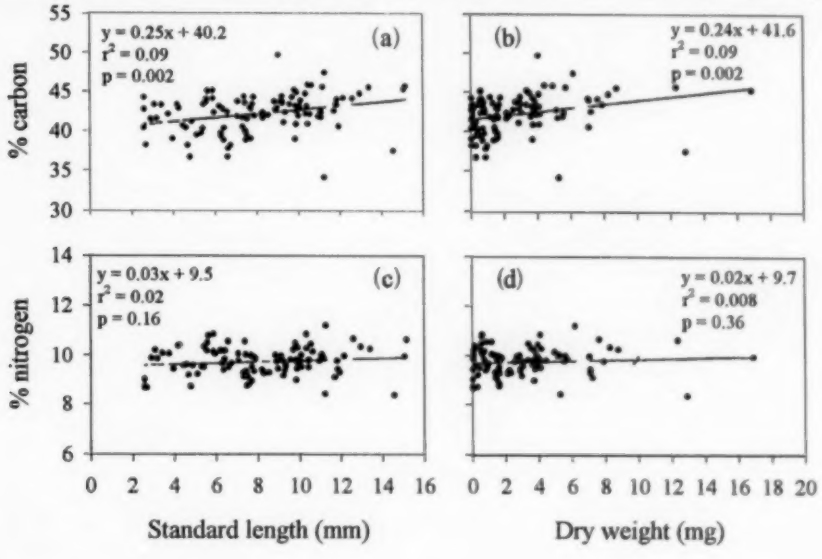

Table 2. Exponents of metabolic decay (c) derived through nonlinear curve fitting of Fry and Arnold's (1982) equation to $\delta^{13}C$ and $\delta^{15}N$ of red drum during dietary switch experiments.

	Carbon				Nitrogen			
	c	SE	r^2	p	c	SE	r^2	p
First feeding (28°C)	–0.96	0.35	0.93	ns	–0.99	0.29	0.96	ns
Settlement size (28°C)	–1.96	0.89	0.56	ns	–1.13	0.25	0.93	ns
Settlement size (24°C)	–1.12	0.32	0.91	ns	–0.94	0.09	0.99	ns

Note: When c = –1, isotopic changes are solely a function of growth (simple dilution model), whereas c less than –1 is indicative of the added effect of metabolic turnover. Standard error values refer to c estimates. Coefficients of determination (r^2) refer to the regression of measured δ values versus those predicted with a simple dilution model and represent the fraction of the observed variability that is explained by growth alone. Values of p were derived using a Student's t test to examine for significant differences between c and –1. ns, not significant.

tions must be met. First, fractionation must be independent of size so that observed patterns in $\delta^{13}C$ and $\delta^{15}N$ of pre- and post-settlement larvae can be attributed to dietary food sources and not to size-related fractionation. For teleosts, some researchers have reported trends in fractionation as a function of size (e.g., Rounick and Hicks 1985; Sholto-Douglas et al. 1991; Hobson and Welch 1995), while others have not found a consistent relationship (Fry and Parker 1979; Fry 1983). Many have relied on field collections (but see Rounick and Hicks 1985), and as noted by the authors, the observed trends could have been confounded by seasonal effects, size-dependent feeding, and fluctuations in the composition of available foods. Variations in tissue lipid content may lead to size-dependent fractionation (DeNiro and Epstein 1977), although the contribution of isotopically lighter lipids to the $\delta^{13}C$ values of whole-fish tissues has been found to be negligible in some cases (Fry and Parker 1979; Hesslein et al. 1993).

Second, in the case of food deprivation, the isotopic composition of larval tissues must not change substantially over the time scale in which isotopic changes in response to a dietary shift are expected to occur. Since fish larvae that are not feeding must rely on resorption of existing tissues to sustain metabolic demands, it was hypothesized that catabolism could result in selective respiration of the lighter carbon isotope (DeNiro and Epstein 1978) or selective excretion of the lighter nitrogen isotope (Hobson et al. 1993). Both processes could result in a shift in isotopic composition over time, confounding the identification of dietary switches following settlement.

Fractionation was examined as a function of size by weaning red drum larvae onto an artificial diet of constant isotopic composition. Despite a slight but significant effect of size on $\delta^{13}C$ fractionation, our results indicate that a value of +1‰ can be considered representative for red drum larvae (4–15 mm SL). Due to an incomplete shift in $\delta^{15}N$ (see Results section), the range of larval sizes used to estimate fractionation was limited to 9–15 mm SL. Nevertheless, no effect of size was detected within that size range and fractionation was +1.6‰.

Fractionation can also be estimated through comparison of the isotopic values of food sources utilized and larvae collected during dietary switch experiments. Fractionation factors ranged between 0.2 and 1.9‰ for carbon and between

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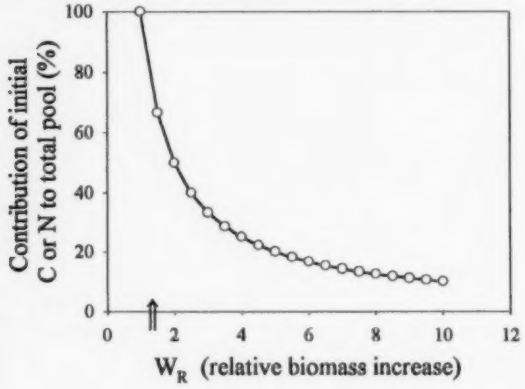
Fig. 6. Estimated dilution of the initial carbon or nitrogen pool by the addition of new biomass during growth. A simple dilution model was utilized. The arrow indicates dietary shift.



1.5 and 4.2‰ for nitrogen (Table 1). These values, and those derived from larvae fed the artificial diet, are within literature values for larval fishes reared in the laboratory ($\delta^{13}C$ fractionation of +2‰ for red drum larvae; Anderson et al. 1988) and collected in the wild (Thayer et al. 1983; Hobson and Welch 1995). They are also within the range of fractionation factors reported for juvenile and adult teleosts (Rounick and Hicks 1985; Sholto-Douglas et al. 1991; Hesslein et al. 1993). Nevertheless, at least part of the variability observed in fractionation values calculated from data collected during dietary switch experiments can arise from variability in the isotopic composition of the rotifer culture, the small number of fish analyzed, and the parameter estimate error associated with δ_{final}. Therefore, we believe that the calculation of fractionation factors based on larger numbers of fish fed a diet of constant isotopic composition is more accurate.

During the 4-day food deprivation period examined, there was no indication of changes in the $\delta^{13}C$ and $\delta^{15}N$ of settlement-size red drum. In a similar experiment with larval krill (*Euphausia superba*), Frazer et al. (1997) found limited effects of starvation on $\delta^{13}C$ and $\delta^{15}N$. Since we did not measure individual weight loss, which would represent a measure of the extent of starvation, it is possible that catabolic processes had not proceeded for a sufficiently long period to result in a shift in isotopic composition. Nevertheless, the lack of a measurable effect of food deprivation on δ values of red drum larvae suggests that it is improbable that its effects could be confounded with a dietary shift in the wild, particularly due to the rapid growth rates exhibited by larvae under natural conditions (Rooker and Holt 1997). In addition, gut content analysis of postsettlement red drum collected in Texas seagrass meadows revealed a low incidence of empty guts in this species, suggesting that long-term food deprivation is not prevalent (Soto et al. 1998).

Contribution of growth and metabolic turnover to isotopic change

Curve-fitted values for the exponent of metabolic decay were about –1 in five out of six dietary switch experiments (except for $\delta^{13}C$ in settlement-size red drum reared at 28°C). Such a value indicates the absence of an effect of metabolic turnover on isotopic composition and implies that patterns of isotopic change are a result of the dilution of an initial carbon or nitrogen pool by the addition of newly deposited biomass. Although metabolic turnover appeared to accelerate the pattern of $\delta^{13}C$ change in settlement-size fish reared at 28°C, the exponent of metabolic decay was not significantly different from –1. This result may be associated with the narrow range in $\delta^{13}C$ values (1.5‰) (Table 1) between the initial and final diet during that dietary switch experiment and consequent large standard error associated with the parameter estimate (Table 2). Nevertheless, due to the overwhelming effect of growth on the isotopic composition of the larvae following dietary shifts, we suggest that, for red drum, patterns of isotopic change in response to dietary shifts can be adequately predicted based on growth rate estimates.

Although studies addressing rates of isotopic change in growing organisms are few, those completed on poikilotherms support this study's finding that biomass gain plays the dominant role. Hesslein et al. (1993) utilized cultured broad whitefish (*Coregonus nasus*) juveniles (initial size 21–210 mm) to examine $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in response to a dietary shift and attributed 90% of the observed isotopic changes to growth. Fry and Arnold (1982) examined $\delta^{13}C$ shifts in juvenile brown shrimp (*Penaeus aztecus*) and found that biomass gain was the primary cause of change in isotopic composition, although the added effect of metabolic turnover was also detected. Likewise, Frazer er al. (1997) examined $\delta^{13}C$ and $\delta^{15}N$ of larval krill and found an effect of metabolism turnover only at the highest of two rearing temperatures. The high contribution of metabolic turnover to isotopic changes in studies with mammals and birds (Tieszen et al. 1983; Hobson and Clark 1992) compared with our own and with those of Fry and Arnold (1982), Hesslein et al. (1993), and Frazer et al. (1997) is probably attributable to the higher basal metabolism of endotherms, which is considerably higher than for poikilotherms such as fish and crustaceans.

Nevertheless, the absence of a detectable effect of nitrogen metabolic turnover in larval fishes was unanticipated because nitrogen cycling is a complex process that is intimately linked with growth (Houlihan et al. 1995*a*), and the rate of protein degradation in marine fish larvae is known to be high (Houlihan et al. 1995*b*). However, in this study and others, metabolic turnover as related to isotopic shifts refers specifically to (*i*) breakdown and (*ii*) replacement of existing body tissues. Hence, metabolic turnover would only accelerate the rate of isotopic change relative to the simple dilution model if the replacement of existing body tissues were accomplished with proteins synthesized from the "new" food source (de novo synthesis). On the other hand, if protein resynthesis from degraded products were high, it is possible that little of the degraded nitrogen pool would be replaced through de novo synthesis. Unfortunately, there is limited information on the mechanisms of protein degradation and fate of degraded products in marine fish larvae (Houlihan et al. 1995*b*). As recently noted by Gannes et al. (1997), the use of stable isotope ratios in ecological studies would be

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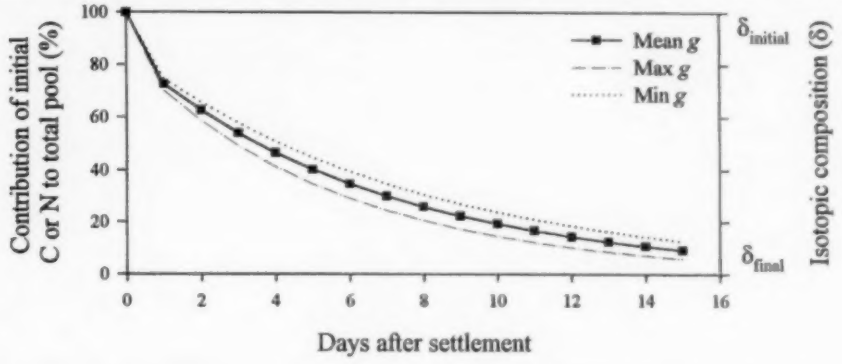
Fig. 7. Estimated dilution of the initial carbon and nitrogen pool expressed as a function of time after settlement for a 7-mm SL red drum larvae. Estimates were derived using instantaneous growth coefficients (g) reported by Rooker and Holt (1997) for postsettlement red drum and an exponent of metabolic decay of –1. Dilution as a function of growth is expressed as the percent contribution of carbon or nitrogen present at the time of settlement to the total and as a function of initial and final isotopic composition.



greatly advanced if the relationships between an animal's diet and the fate of assimilated components and synthesized products were elucidated and the biochemical origin of fractionation determined.

Isotopic change in natural populations

Based on the absence of an effect of metabolic turnover, it can be estimated that red drum larvae should reflect the isotopic composition of a new food (<20% of the total carbon or nitrogen attributable to that present at settlement) after a sixfold biomass increase. It follows that animals exhibiting fast growth rates will display faster changes in isotopic composition in response to a dietary shift than animals exhibiting little or no growth. For comparative purposes, the exponential growth model used by Rooker and Holt (1997, eq. 7) was curve-fitted to the length at age data obtained during dietary switch experiments. The instantaneous growth coefficients (g) obtained ranged between 0.06 and 0.09·day^{-1}, similar to those of previous reports for laboratory-reared red drum (0.04–0.08·day^{-1}) (e.g., Holt 1993). The values of g reported by Rooker and Holt (1997) for newly settled red drum were generally lower (about 0.04–0.06·day^{-1}). Hence, the interpretation of isotopic data collected for natural populations of newly settled fish relies on the adequacy of growth estimates.

Based on the mean g value reported by Rooker and Holt (1997), newly settled red drum should exhibit a shift from the isotopic signature characteristic of a planktonic food web in as little as 1–2 days after settlement and should exhibit an isotopic signature resembling that of demersal food sources after 10 days. Variability in growth rates was estimated to account for about a 10% difference in the estimated contribution of the initial carbon or nitrogen pool to the total on any day after settlement. Although the use of average growth rates is probably adequate, the effect of growth rate variability (individual and population) on isotopic changes in response to a dietary shift is clearly of importance.

Potential for field application

Fry (1983) utilized $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ to trace the migration of shrimp and fish species between estuarine and offshore habitats. More recently, Hentschel (1998) analyzed the $\delta^{13}C$ of wild-caught deposit-feeding polychaetes and noted differences between the isotopic composition of pre- and post-settlement larvae as well as ontogenetic dietary shifts in postsettlement larvae and juveniles. Similarly, stable isotope ratios may serve as tracers of recent settlement of red drum and other fish species. A key requirement is that sufficient differences in food sources and (or) food web structure among planktonic and demersal habitats exist. Ideally, newly settled fish should reflect a "planktonic" isotopic composition, whereas nursery habitat residents should have one that resembles benthic carbon sources or some mixture of planktonic and benthic carbon sources that is isotopically distinguishable from a purely planktonic signature (e.g., France 1995). Red drum feed on numerous types of organisms, including various types of copepods and copepod nauplii, mysid shrimp, dinoflagellates, bivalve larvae, and invertebrate eggs and nauplii (Soto et al. 1998; Holt and Holt 1999). The isotopic composition of red drum tissues will integrate that of all the food items ingested and assimilated and is thus likely to depend on the local food web structure (e.g., Deegan et al. 1990).

Typical $\delta^{13}C$ values for phytoplankton are –18 to –24‰ (Fry and Sherr 1984). In our study area in Texas, recently settled red drum inhabit seagrass meadows (Holt et al. 1983). A variety of primary producer groups, including seagrasses ($\delta^{13}C$ = –8.3 to –12.5‰; McMillan et al. 1980), macroalgae ($\delta^{13}C$ = –2 to –8‰; Fry and Sherr 1984), and epiphytes ($\delta^{13}C$ = –13 to –17‰; Kitting et al. 1984), can influence the isotopic composition of food sources available to red drum larvae. Fry and Parker (1979) compared $\delta^{13}C$ values for a variety of animals collected in Texas seagrass meadows and the nearshore Gulf of Mexico and found that fish collected in seagrass habitat tended to be about 5‰ heavier. On the other hand, for $\delta^{15}N$ to be useful as a tracer of recent settlement, it is likely that larvae would have to undergo a distinct trophic shift as a result of settlement.

The ability to detect newly settled red drum will depend on the absolute difference in the $\delta^{13}C$ and (or) $\delta^{15}N$ between pre- and post-settlement larvae. The smaller the difference, the greater the difficulty in resolving and identifying a dietary switch and the smaller the temporal window of detec-

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tion. Assuming adequate differences in $\delta^{13}C$ and $\delta^{15}N$ of food sources in the plankton and nursery habitat (e.g., 5‰), and given the growth rates of postsettlement red drum, newly settled larvae could be detected up to 4–6 days after settlement.

An additional requirement of a useful tool for settlement studies is that it must be applicable on an individual basis. Due to recent analytical advances in mass spectrometry, $\delta^{13}C$ and $\delta^{15}N$ can be easily measured using relatively small quantities of tissue. Depending on the size and weight of the target species at settlement, the isotopic composition of individual fish larvae can be easily determined (e.g., red drum larvae >5 mm SL or about 400 µg DW). Hence, the utilization of stable isotope analysis to identify new settlers could be accomplished individually.

Conclusions

The study of the fine-scale temporal dynamics of settlement requires the identification of chemically or morphologically based characters that can be used to identify new settlers over short time intervals. Given adequate differences in food webs among habitats, new settlers should be distinguishable from previous arrivals to nursery habitat over a time scale of days. Comparison of $\delta^{13}C$ and $\delta^{15}N$ values of planktonic larvae with those of postsettlement larvae could be used to estimate the proportion of new settlers on a size-class and date-specific basis. Furthermore, the time since settlement could be estimated using growth rate estimates (Fry and Arnold 1982). The utilization of stable isotope ratios as tracers of individual recruitment to nursery areas could allow for increased temporal resolution of settlement patterns, therefore contributing to the understanding of the early life dynamics of marine fish species.

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References

Anderson, R.K., Dickson, M.L., Holt, G.J., Ritz, D., and Parker, P.L. 1988. The use of stable isotope tracers in red drum (*Sciaenops ocellatus*) culture. Contrib. Mar. Sci. **30**: 87–92.

Arnold, C.R. 1988. Controlled year-round spawning of red drum *Sciaenops ocellatus* in captivity. Contrib. Mar. Sci. **30**(Suppl.): 65–70.

Boehlert, G.W., and Mundy, B.C. 1988. Roles of behavior and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. Am. Fish. Soc. Symp. **3**: 51–67.

Deegan, L.A., Peterson, B.L., and Portier, R. 1990. Stable isotopes and celullase activity as evidence for detritus as a food source for juvenile Gulf Menhaden. Estuaries. **13**: 14–19.

DeNiro, M.J., and Epstein, S. 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. Science (Washington, D.C.), **197**: 261–263.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**: 495–506.

Doherty, P.J., and Williams, D.McB. 1988. The replenishment of marine fish populations. Oceanogr. Mar. Biol. Annu. Rev. **26**: 487–551.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to pelagic algae: food web implications. Mar. Ecol. Prog. Ser. **124**: 307–312.

Frazer, T.K., Ross, R.M., Quetin, L.B., and Montoya, J.P. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. J. Exp. Mar. Biol. Ecol. **212**: 259–275.

Fry, B. 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. Fish. Bull. U.S. **81**: 789–801.

Fry, B., and Arnold, C. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp *(Penaeus aztecus)*. Oecologia,. **54**: 200–204.

Fry, B., and Parker, P.L. 1979. Animal diet in Texas seagrass meadows: $\delta^{13}C$ evidence for the importance of benthic plants. Estuarine Coastal Mar. Sci. **8**: 499–509.

Fry, B., and Sherr, E.X. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. **27**: 15–47.

Gannes, L.Z., O'Brien, D.M., and Martinez del Rio, C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology, **78**: 1271–1276.

Grover, J.J., Eggleston, D.B., and Shenker, J.M. 1998. Transition from pelagic to demersal phase in early-juvenile Nassau grouper, *Epinephelus striatus*: pigmentation, squamation, and ontogeny of diet. Bull. Mar. Sci. **62**: 97–111.

Hentschel, B.T. 1998. Intraspecific variations in $\delta^{13}C$ indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology, **79**: 1357–1370.

Hesslein, R.H., Capel, M.J., Fox, D.E., and Hallard, K.A. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the Lower Mackenzie River Basin, Canada. Can. J. Fish. Aquat. Sci. **48**: 2258–2265.

Hesslein, R.H., Hallard, K.A., and Ramlal, P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can. J. Fish. Aquat. Sci. **50**: 2071–2076.

Hobson, K.A., and Clark, R.G. 1992. Assessing avian diets using stable isotopes. I: Turnover of ^{13}C in tissues. Condor, **194**: 181–188.

Hobson, K.A., and Welch, H.E. 1995. Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis. Can. J. Fish. Aquat. Sci. **52**: 1195–1201.

Hobson, K.A., Alisaiskas, R.T., and Clark, R.G. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor, **95**: 388–394.

Holt, G.J. 1992. Experimental studies of feeding in larval red drum. J. World Aquacult. Soc. **23**: 265–270.

Holt, G.J. 1993. Feeding larval red drum on artificial diets in a closed recirculating water system. J. World Aquacult. Soc. **24**: 225–230.

Holt, G.J., and Holt, S.A. 1999. Vertical distribution and the role of physical processes in the feeding dynamics of two larval sciaenids,

© 2000 NRC Canada

Sciaenops ocellatus and *Cynoscion nebulosus*. Mar. Ecol. Prog. Ser. In press.

Holt, G.J., Johnson, A.G., Arnold, C.R., Fable, W.A., and Williams, T.D. 1981*a*. Description of eggs and larvae of laboratory reared red drum, *Sciaenops ocellata*. Copeia, **4**: 751–756.

Holt, G.J., Godbout, R., and Arnold, C.R. 1981*b*. Effects of temperature and salinity on eggs hatching and larvae survival of red drum *Sciaenops ocellata*. Fish. Bull. U.S. **79**: 569–573.

Holt, S.A., Kitting, C.L., and Arnold, C.R. 1983. Distribution of red drum among different seagrass meadows. Trans. Am. Fish. Soc. **112**: 267–271.

Houlihan, D.F., Carter, C.G., and McCarthy, I.D. 1995*a*. Protein synthesis in fish. *In* Biochemistry and molecular biology of fishes. *Edited by* P.W. Hochachka and T.P. Mommsen. Elsevier Science, New York. pp. 191–220.

Houlihan, D.F., Pedersen, B.H., Steffensen, J.F., and Brechin, J. 1995*b*. Protein synthesis, growth and energetics of larval herring (*Clupea harengus*) at different feeding regimes. Fish Physiol. Biochem. **14**: 195–208.

Kitting, C.L., Fry, B., and Morgan, M.D. 1984. Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. Oecologia, **62**: 145–149.

Leis, J.M. 1991. The pelagic stage of reef fishes: the larval fish biology of coral reefs. *In* The ecology of fishes in coral reefs. *Edited by* P.F. Sale. Academic Press, San Diego, Calif. pp. 193–230.

McMillan, C., Parker, P.L., and Fry, B. 1980. $\delta^{13}C$ ratios in seagrasses. Aquat. Bot. **9**: 237–249.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**: 293–320.

Roelke, L.A., and Cifuentes, L.A. 1997. Uses of stable isotopes to assess groups of king mackerel, *Scomberomous cavalla*, in the Gulf of Mexico and southeastern Florida. Fish. Bull. U.S. **95**: 540–551.

Rooker, J.R., and Holt, S.A. 1997. Utilization of subtropical seagrass meadows by newly settled red drum *Sciaenops ocellatus*: patterns of distribution and growth. Mar. Ecol. Prog. Ser. **158**: 139–149.

Rooker, J.R., Holt, S.A., Soto, M.A., and Holt, G.J. 1998. Post-settlement patterns of habitat use by sciaenid fishes in subtropical seagrass meadows. Estuaries, **21**: 318–327.

Rounick, J.S., and Hicks, B.J. 1985. The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biol. **15**: 207–214.

Sholto-Douglas, A.D., Field, J.G., James, A.G., and van der Merwe, N.J. 1991. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish. Differences between fish muscle and bone collagen tissues. Mar. Ecol. Prog. Ser. **78**: 23–31.

Soto, M.A., Holt, G.J., Holt, S.A., and Rooker, J. 1998. Food habits and dietary overlap of newly settled red drum (*Sciaenops ocellatus*) and Atlantic croaker (*Micropogonias undulatus*) from Texas seagrass meadows. Gulf Res. Rep. **10**: 41–55.

Thayer, G.W., Govoni, J.J., and Connally, D.W. 1983. Stable isotope ratios of the planktonic food web in the northern Gulf of Mexico. Bull. Mar. Sci. **33**: 247–256.

Thorrold, S.R., Jones, C.M., and Campana, S.E. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol. Oceanogr. **42**: 102–111.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia, **57**: 32–37.

Victor, B.C. 1991. Settlement strategies and biogeography of reef fishes. *In* The ecology of fishes in coral reefs. *Edited by* P.F. Sale. Academic Press, San Diego, Calif. pp. 231–260.

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Identification of gender and reproductive maturity in the absence of gonads: muscle tissue levels of sex steroids and vitellogenin in gag (*Mycteroperca microlepis*)

Scott A. Heppell and Craig V. Sullivan

Abstract: Gag (*Mycteroperca microlepis*) are often landed eviscerated, so their gonads are not available for histological verification of gender and stage of maturity. Information on gender is particularly important for the management of hermaphroditic grouper, where increased mortality through fishing can directly affect sex ratio and therefore the reproductive capacity of the population. Alternative techniques for evaluating fish gender and maturity therefore need to be developed for gag and other grouper. We utilized sensitive immunoassays to measure levels of the sex steroids estradiol-17β (E_2), testosterone (T), and 11-ketotestosterone (11KT) and the egg-yolk precursor vitellogenin (VTG) in gag on a quarterly basis. Plasma and muscle levels of E_2, T, and VTG in females were lowest during summer, rising in winter to reach peak values in spring. During winter and spring, plasma and muscle levels of 11KT were significantly higher in males than in adult females or immature fish. Combined measurement of VTG and 11KT in gag muscle proved useful for differentiating between males, adult females, and immature fish between December and April, the period of active gonadal recrudescence. This technique should prove useful in cases where fishery data are primarily collected through port sampling and gonads are not available for analysis.

Résumé : La badèche baillon (*Mycteroperca microlepis*) est souvent débarquée éviscérée, de telle sorte que ses gonades ne peuvent pas servir à la vérification histologique du genre et du stade de maturité. Les données sur le genre sont particulièrement importantes pour la gestion des badèches hermaphrodites, car un accroissement de la mortalité par la pêche peut affecter directement le taux de masculinité, et donc la capacité de reproduction de la population. Il faut donc développer d'autres techniques d'évaluation du genre et du stade de maturité pour la badèche baillon et d'autres espèces de ce genre. Nous avons utilisé des immunoessais sensibles pour doser le taux des hormones stéroïdiennes sexuelles (17β-oestradiol (E_2), testostérone (T) et 11-cétotestostérone (11KT)) et le précurseur du vitellus, la vitellogénine (VTG), chez la badèche baillon sur une base trimestrielle. Le taux de E_2, de T et de VTG dans le plasma et les muscles chez les femelles était au plus bas pendant l'été, et à la hausse en hiver pour atteindre un pic au printemps. Pendant l'hiver et le printemps, le taux de 11KT dans le plasma et les muscles était significativement plus élevé chez les mâles que chez les femelles adultes ou les poissons immatures. Les mesures combinées de VTG et de 11KT dans les muscle des badèches baillon se sont révélées utiles pour différencier les mâles, les femelles adultes et les individus immatures, entre décembre et avril, période de reprise de l'activité gonadique. Cette technique devrait servir dans les cas où les données sur la pêche proviennent surtout de l'échantillonnage au port et où les gonades ne sont pas disponibles aux fins d'analyse.

[Traduit par la Rédaction]

Introduction

The gag (*Mycteroperca microlepis*) is a temperate marine serranid native to the southeast Atlantic coast of the United States and the Gulf of Mexico. This is an economically important species subject to intense pressure from both commercial fishers and recreational anglers (Manooch and Haimovici 1978; National Marine Fisheries Service, Fisheries Statistics and Economics Division, personal communication) to the extent that gag stocks have diminished much over the last decade (Collins et al. 1987; McGovern et al. 1998). Gag are protogynous hermaphrodites and there is evidence that the larger and more aggressive males have been selectively removed from populations fished while in spawning aggregations (Gilmore and Jones 1992; Coleman et al. 1996). Accordingly, evaluation of reproductive parameters including adult sex ratio, size- and age-at-maturity, and fecundity is critical to scientific management of the gag fishery for sustained yields (Coleman et al. 1996; McGovern et al. 1998).

Especially needed for rational management of gag and other grouper fisheries is knowledge of the stock composition with respect to sex and maturational status. This information is used to construct a maturity schedule that estimates the proportion of fish in each age- or size-class

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that are sexually mature. The maturity schedule, in conjunction with information on stock size and weight-specific fecundity of females, is used to predict the outcome of fishing pressure and develop fishery management policies (Pitcher and Hart 1982; Getz and Haight 1989). The conventional approach to assessing fish gender and maturity involves histological analysis of the gonads. However, this approach cannot always meet the needs of grouper biologists.

In the grouper fishery, fish are typically eviscerated at capture to maintain product quality and optimize efficient use of ice and space on fishing vessels, which may remain at sea for several days at a time. Therefore, sampling grouper for gonadal tissue when they are landed is not feasible for fishery-dependent assessment of their reproductive demographics. An emergency rule was recently enacted to require some vessels to land gag intact so that their gender and reproductive condition can be evaluated (McGovern et al. 1998), but this measure is controversial and places considerable burden on commercial fishers. The alternative approach, collecting fishery-independent data on intact fish caught during scientific expeditions, is logistically challenging and very costly. Using this approach, it is impractical to evaluate maturity schedules annually, so resulting management models may be somewhat static and ignore short-term effects of fishing pressure or environmental perturbation. Finally, histological evaluation of fish maturity is laborious, expensive, and slow to generate usable data.

Clearly, it would benefit grouper biologists and fishers if a practical biochemical test could be developed to assess the gender and maturational status of eviscerated fish. Such tests have been developed for intact fishes. Most tests focus on detection of estrogen, androgens, or the egg-yolk protein precursor protein vitellogenin (VTG) in the circulation (Fostier et al. 1983; Gordon et al. 1984; Pankhurst and Carragher 1991; Specker and Anderson 1994; Johnson et al. 1998). It has also been demonstrated that VTG can be measured in fish scale mucus (Gordon et al. 1984; Kishida et al. 1992). Detection of sex-specific compounds outside the bloodstream is advantageous for fisheries management applications because fish that have been eviscerated or otherwise processed at sea lack appreciable amounts of blood for analyses.

In the present study, we evaluated levels of estrogen (estradiol-17β (E_2)), androgens (11-ketotestosterone (11KT) and testosterone (T)), and VTG in the skeletal muscle of gag to assess the potential utility of these compounds as markers of gender and maturity for this species. Muscle was targeted because it is the primary fishery product that is landed and so is easy to collect for analyses. Furthermore, samples of muscle tissue could be used for simultaneous population genetics studies and forensic identification of species. VTG was measured using a sensitive enzyme-linked immunosorbent assay (ELISA) that we recently developed for gag VTG (Heppell and Sullivan 1999), and sex steroids were quantified using established radioimmunoassays (RIAs). Levels of VTG and sex steroids were measured on a quarterly basis throughout the year in muscle of male and female gag at various reproductive stages, and the values for muscle were compared with blood plasma levels of these compounds in the same fish. Plasma levels of the sex steroids and VTG in gag were calibrated to specific stages of ovarian maturation in a prior investigation (Heppell and Sullivan 1999).

Materials and methods

Sample collection

Male and female gag at various stages of maturity were collected between December 1994 and June 1996 by hook and line, fish traps, and spearfishing from the Gulf of Mexico off the Florida coast and from the western Atlantic Ocean between Cape Hatteras, North Carolina, and Cape Canaveral, Florida. Sampling occurred on a quarterly basis, designed to represent four periods of reproductive maturation: summer (quiescence), fall (early maturation), winter (midmaturation), and spring (final maturation and spawning). The actual dates (inclusive, pooled for both years) during which sampling occurred in these periods were as follows: summer, 11 April to 27 June; fall, 13 September to 27 September; winter, 11 November to 29 December; spring, 24 February to 22 March. The total length (TL, millimetres) of each fish was measured and body weight (grams) was measured when possible. The fish were then bled by caudal puncture into heparinized syringes. The blood was treated with heparin and the protease inhibitor aprotinin, and the plasma was separated into small (~200 μL) aliquots that were snap frozen on dry ice and stored at –80°C until being analyzed for VTG and sex steroids (Tao et al. 1993; Heppell and Sullivan 1999). After bleeding the fish, their gonads were collected and immediately preserved in 10% neutral buffered formalin for a minimum of 2 weeks at 0–4°C before processing for histological analysis. For fish with gonads larger than about 2 cm^3, a tissue sample was collected from the anterior portion of the left (larger) lobe of the gonad. Samples of skeletal muscle were obtained from each fish from the abdomen just anterior to the urogenital pore. Muscle samples were frozen immediately on dry ice for transportation to the laboratory and subsequent storage at –80°C for later analyses of sex steroid and VTG levels. Sagittal otoliths were removed from each fish, dried, mounted on glass microscope slides with thermoplastic, and sectioned through the nucleus on an Isomet low-speed saw (Beuhler) using a diamond blade. Sections were then affixed to glass slides and dense bands were scored as annual growth marks used to verify the age of each animal (Collins et al. 1987; Johnson et al. 1993).

Gonadal histology

The fixed tissue samples were dehydrated in ethanol, infiltrated with and embedded in catalyzed JB-4 glycolmethacrylate, sectioned at 3.5 μm on a rotary microtome using glass knives, mounted on glass slides, stained with hematoxylin, and counterstained with eosin (Jackson and Sullivan 1995). Gonad sections were viewed under a compound light microscope at either 40 or 100× magnification and classified as to stage of maturity. The histological stages of ovarian maturity in gag were illustrated and described in detail in a prior report (Heppell and Sullivan 1999). They are named according to maturational processes evident in the largest oocytes present as follows: stage 0, immature; stage 1, primary growth; stage 2, early secondary growth; stage 3, early vitellogenesis; stage 4, late vitellogenesis; stage 5, final maturation and ovulation. These stages were previously used to construct a maturity schedule characterizing the size-at-maturity (TL) of the female gag and to correlate their plasma VTG and sex steroid levels with specific stages of ovarian maturation (Heppell and Sullivan 1999). An insufficient number of males were collected to allow rigorous analyses of male reproductive function, and only four stages were used to classify the developmental state of testicular tissue as follows: stage 1, undeveloped; stage 2, early spermatogenesis; stage 3, late spermatogenesis; stage 4, spermiation.

Enzyme-linked ELISA of VTG

Plasma VTG levels were measured in a homologous, competitive ELISA utilizing a polyclonal antiserum to gag VTG (gVTG)

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with purified gVTG as the standard (Heppell and Sullivan 1999). Prior to analysis in the VTG ELISA, plasma was diluted 1:2000 (v/v) in PBST (0.01 M NaH_2PO_4/Na_2HPO_4, 0.15 M NaCl, 0.05% Tween-20, pH 7.4) containing 2.5% (v/v) normal goat serum (PBST-NGS). For analysis of VTG concentrations in muscle, 500 mg of muscle tissue from each fish was finely chopped with a razorblade, mixed with 500 µL of PBS (0.01 M NaH_2PO_4/Na_2HPO_4, 0.15 M NaCl, pH 7.4) in a 12 × 75 mm glass culture tube, and homogenized on ice using a Tissue Tearor™ motorized homogenizer (model 985-370; Biospec Products, Inc.) at setting 2–3 for 20–30 s. The resulting homogenate was centrifuged at 12 500 × *g* for 10 min and the supernatant was collected and recentrifuged. The final supernatant was collected as the muscle extract, diluted in PBST, and analyzed in the VTG ELISA as described above for plasma samples. VTG levels in the muscle tissue extract were expressed as micrograms of VTG per gram wet weight of tissue.

Steroid RIA

Blood plasma concentrations of E_2, T, and 11KT were measured in 20 µL of gag plasma using established RIAs (Woods and Sullivan 1993). Plasma for E_2 analysis was double extracted in ethyl ether, dried under a stream of nitrogen gas at 37°C, and then resuspended in buffer for assay. Plasma for T and 11KT analysis was triple extracted prior to being dried down and resuspended in assay buffer.

Parallelism over the working range of the RIAs of circulating steroids to authentic purified steroid standards was demonstrated by serially diluting extracts of plasma samples pooled from several mature male or female gag in assay buffer before RIA. Counts of radioactivity were converted to B/B_0 values and logit transformed ($\log[B/B_0/(1 - B/B_0)]$) to linearize results.

Extraction efficiency was verified for each steroid by spiking triplicate 50-µL samples of plasma or assay buffer (control) with approximately 3000 counts per min (cpm) of radioactively labeled steroid. The spiked samples were incubated at room temperature for 1 h, after which 20-µL aliquots of each sample were extracted, dried in a water bath at 37°C under a stream of nitrogen gas, and resuspended in assay buffer. The resuspended extracts were brought up to 900 µL total volume in assay buffer, decanted into vials containing 3 mL of scintillation cocktail, and read for 5 min on a liquid scintillation counter. Percent steroid recovery was measured as (cpm in the extracted plasma sample/cpm in the control sample) × 100.

Steroids in extracts of 500 mg of muscle tissue were quantified by RIA. Muscle samples were thawed, finely chopped with a razorblade, homogenized with 1.5 mL of methanol in a 12 × 75 mm glass culture tube using the Tissue Tearor™ motorized homogenizer at setting 2–3 for 20–30 s, and then centrifuged at 1000 × *g* for 10 min. The aqueous lower phase and pelleted insoluble material were snap frozen in liquid nitrogen and the methanol extract was decanted into a new 12 × 75 mm tube. The lower phase and pellet were thawed, vortex mixed with another 1.5-mL aliquot of methanol, and recentrifuged. After snap freezing the sample, the second methanol extract was combined with the first and dried down in a water bath at 37°C under a stream of nitrogen gas. The remaining fraction was double extracted with two 1.5-mL volumes of ethyl ether to remove any remaining particulates and the combined ether extracts were dried at 37°C under nitrogen, resuspended in 400 µL of assay buffer, and split into two 12 × 75 mm glass culture tubes. The duplicate samples were analyzed for steroid content by RIA as described above for plasma.

The efficiency of extraction of steroids from muscle tissue was estimated using a sample of muscle pooled from several adult gag to which tritiated steroids standards were added (spiked). The muscle was homogenized as described above and approximately 3000 cpm of radiolabeled E_2, T, or 11KT was added to triplicate 200-µL samples of the homogenate. The spiked homogenates were incubated at 4°C for 1 h and then extracted as above and resuspended in 200 µL of assay buffer for RIA. Control samples were prepared by adding 3000 cpm of radiolabeled steroid to 200 µL of assay buffer, followed by methanol and ether extraction and resuspension in 200 µL of assay buffer for RIA. Percent steroid recovery from muscle was measured as (cpm in the extracted plasma sample/cpm in the control sample) × 100.

Parallelism over the working range of the RIAs of steroids in muscle extracts to authentic steroid standards was demonstrated as described for plasma by serially diluting the extracts in assay buffer before RIA.

Statistical analyses

Statistical analyses were performed using the Statistica™ statistical software package (Statsoft, Inc.) or the Microsoft Excel Statistical Analysis Toolpack (Microsoft Corp.). Statistical significance was accepted at $p \leq 0.05$ for all tests. Parallelism in the RIAs for plasma and muscle extract was verified using an analysis of covariance (ANCOVA) *F* test on mean squares to evaluate similarity of slopes between the standard curve for each steroid and the sample dilution series. Normal distribution and homogeneity of variance were confirmed prior to application of ANCOVA and chi-square analyses. For those data for which nonnormality was demonstrated, the nonparametric Mann–Whitney *U* statistic was used.

Results

Experimental animals

Based on previously published maturity schedules for this species (Collins et al. 1987; McGovern et al. 1998), gag smaller than 600 mm TL were considered unlikely to be sexually mature and so were not analyzed with respect to histological maturation of their gonads. Of the 277 fish captured in this study, 123 were females larger than 600 mm TL, subdivided into each quarter as follows: spring (n = 44), summer (n = 12), fall (n = 21), and winter (n = 46). Of the 90 fish collected during winter and spring that were greater than 600 mm TL, 46 were observed to be undergoing maturation based on the presence of vitellogenic oocytes (oocyte stage ≥ 3) in their ovaries. The smallest individual observed to be maturing in this study was 695 mm TL. Only four males were collected in the course of this study, ranging in size from 890 to 1280 mm TL. Because of the small number of males collected, all during the spring quarter, statistical analyses of changes in reproductive parameters (gonadal histology, plasma and muscle sex steroid and VTG levels) were not performed for males. Only one fish was captured while undergoing sexual transition from female to male, during the winter quarter. Winter through spring was identified as the period of maximum gonadal (oocyte) growth and maturation in females. The apparent bimodal distribution evident for the winter and spring samples (Fig. 1) is an artifact of the fish collection process. The larger animals were collected in the commercial fishery, which is subject to a 20-in. minimum size limit, whereas the smaller fish were obtained on fishery-independent scientific expeditions not limited by this constraint.

The length–weight relationship for this data set demonstrates a good correlation between linear growth and volumetric weight gain. When modeled using the formula weight = aL^b, the returned parameters are $a = 2.03 \times 10^{-5}$

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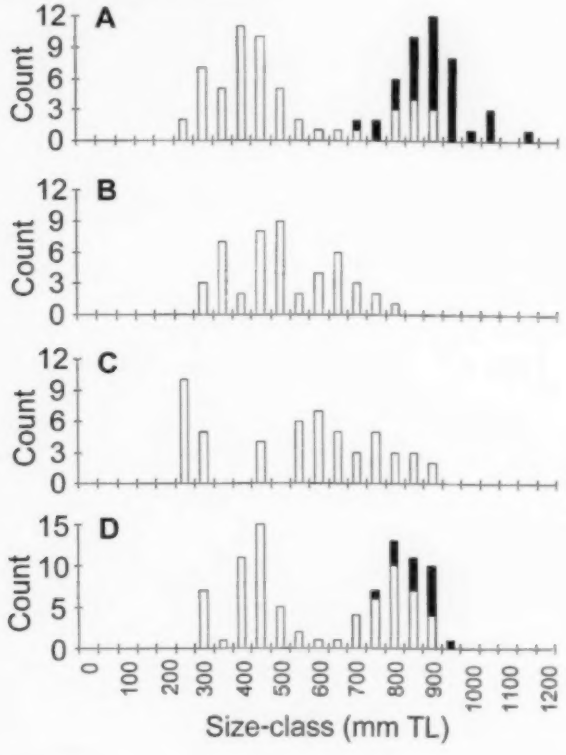
Fig. 1. Length distribution and maturity status of female gag captured during (A) spring, (B) summer, (C) fall, and (D) winter 1994 and 1995. Open bars indicate immature females and solid bars represent mature females (ovarian stage ≥ 3). The bimodal distribution of sizes reflects fishery-dependent versus fishery-independent sampling and differences in sampling gears (see Results, Experimental animals).



and $b = 2.87$. Estimates of the von Bertalanffy growth parameters ($L_t = L_{inf}\ [1 - e^{-k(t-t_0)}]$), constructed from average length-at-age data for this sample set (with age 0 fish excluded), were $L_{inf} = 1187$ mm TL, $k = 0.17$, and $t_0 = -1.09$.

Gonadal histology

As noted, only fish greater than 600 mm TL were evaluated with respect to gonadal histology. None of these fish were females with stage 0 (immature) ovaries. Females with stage 0 and 1 ovaries, containing only primary growth oocytes and (or) oogonia, were captured in all quarters of the year, and all females (>600 mm TL) captured during the summer and fall had ovaries at stage 1 of maturation (see Table 1). Females with ovaries at maturity stages 2 (early secondary growth), 3 (early vitellogenesis), and 4 (late vitellogenesis) were captured in both winter and spring, predominating during the spring quarter collections. Females with stage 5 ovaries, those undergoing final oocyte maturation or ovulation, were observed only in the spring quarter collections. Females captured at stage 4 of ovarian maturation had primary growth oocytes, early secondary growth oocytes, and early vitellogenic oocytes as well as late vitellogenic oocytes in their ovaries (Heppell and Sullivan 1999). Females captured at stage 5 of ovarian maturation had oocytes at all stages of development present in their ovaries, including preovulatory follicles (Fig. 2A). All four males collected during spring were mature animals (stage 4) with well-developed testes containing germ cells at all stages of development, including mature sperm (Fig. 2B). The one transitional animal had developing sperm ducts as well as primary growth oocytes present in its gonad (Fig. 2C). Based on the maturity classification schemes used for males and females, it had a stage 1 ovary and a stage 3 testis.

Table 1. Sample size for each stage of ovarian growth by quarter.

Quarter	Ovarian stage	*n*
Spring	0	32
	1	11
	2	12
	3	1
	4	23
	5	8
Summer	0	15
	1	31
Fall	0	18
	1	35
Winter	0	40
	1	21
	2	13
	3	12
	4	2

Steroid and VTG analyses

In our prior research, the VTG ELISA was developed and validated for use with gag blood plasma, and specific levels of circulating VTG were associated with the various stages of ovarian maturity in gag (Heppell and Sullivan 1999). The upper and lower limits of detection for the ELISA (B/B_0 = 10 and 90%, respectively) were 1452 and 19 ng VTG/mL, respectively, with the midpoint of the assay falling at 165 ng VTG/mL. In general, plasma VTG levels are nondetectable (<19 ng/mL) at stage 2 or earlier stages of ovarian development, low (~0.6 mg/mL) at stage 3, reach peak values (~3.0 mg/mL) at stage 4, and decrease slightly by stage 5.

In the present study, changes over the four quarters of the year in circulating and muscle VTG and steroid levels were evaluated in female gag. Our goals were to (*i*) correlate seasonal changes of circulating VTG and steroid levels with muscle levels of these compounds and (*ii*) evaluate when during the year the muscle analyses might be applicable as tests for gag gender and maturity. During the summer and fall quarters, all females greater than 600 mm TL were used in the analysis, as all had ovaries at the same stage of maturation (stage 1). As noted, females judged to be maturing based on gonadal histology (ovarian stage > 3) were captured only during winter and spring. For these quarters, only maturing females were used in the steroid and VTG analyses.

VTG was virtually nondetectable in blood plasma from females caught during summer and was not detected in fish collected during fall (Fig. 3A). The slight elevation of VTG

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Fig. 2. Photomicrographs of some histological stages of gonadal maturation in gag. (A) Mature ovary from a stage 5 female (magnification 40×, bar = 240 μm). Preovulatory follicles contain the largest oocytes, which are filled with a homogeneous mass of transparent yolk. Such oocytes are present in females ready to ovulate and spawn. Also present are oocytes in primary growth and early secondary growth as well as oocytes undergoing vitellogenic growth and atresia. yg, yolk granules; gv, germinal vesicle; pof, preovulatory follicle; at, atretic follicle. (B) Mature testis from a stage 4 male (magnification 100×, bar (shown in Fig. 2A) = 115 μm). (C) Gonad from the fish captured while undergoing sex change (magnification 100×, bar (shown in Fig. 2A) = 115 μm). Primary growth oocytes are evident as well as germ cells at various stages of spermatogenesis.

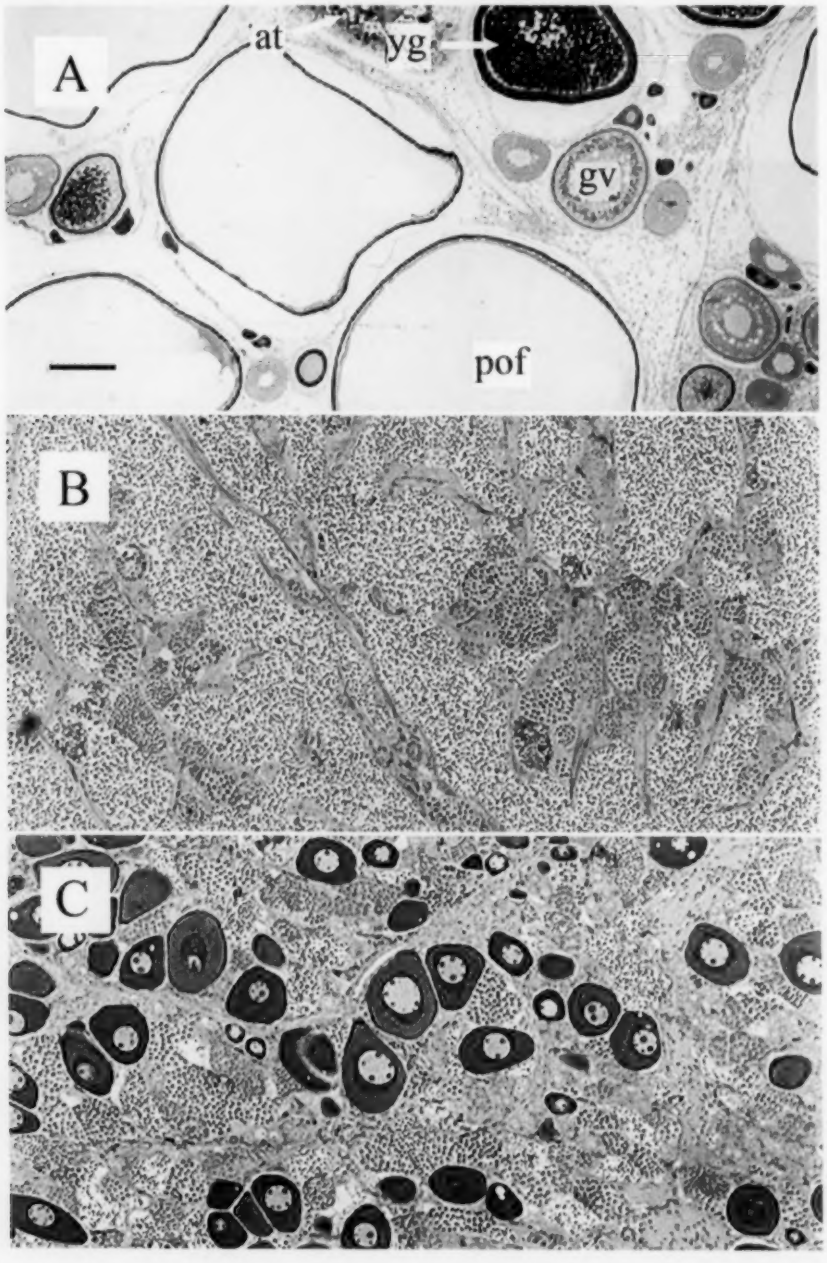

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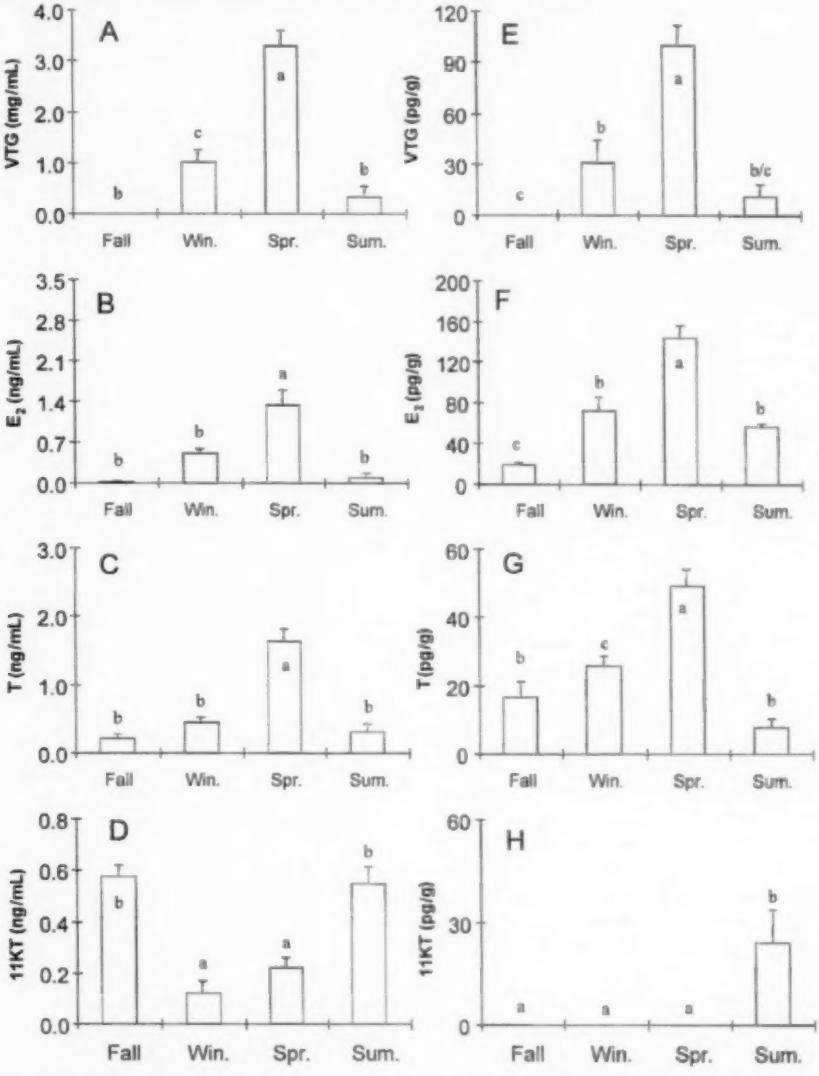
Fig. 3. Seasonal changes in VTG and reproductive steroids (E_2, T, and 11KT) as measured in (A–D) blood plasma and (E–H) extracts of muscle tissue from female gag. Open bars represent the mean value for several fish and vertical brackets indicate SEM. Sample sizes for the four sampling periods were $n = 9$ for fall, $n = 12$ for winter, $n = 28$ for spring, and $n = 9$ for summer. Shared lowercase letters above the bars represent nonsignificant differences (Mann–Whitney U, $p > 0.05$)



above baseline reported during summer is due to the effects of two fish, both of which were collected in early April, probably just as they were completing spawning. Circulating VTG was present at low levels (~0.9 mg/mL) in fish caught during winter. It was first detected in females caught in early December and reached peak values (~4.0 mg/mL) in females caught during spring. The last sampling date during either year on which VTG was detected in females was 15 April. VTG was not detected in the one fish caught while undergoing sexual transition from female to male. We have previously reported that, using this ELISA, VTG cannot be detected in plasma from immature or mature male gag, nor is it usually detectable in plasma from previtellogenic adult females (ovarian stage < 3).

To validate the RIAs for use with gag, the efficiency of steroid extraction from plasma was verified and it was confirmed that gag plasma diluted parallel to the RIA standard curves. Extraction efficiencies for triplicate samples of plasma that had been spiked with tritiated E_2, T, or 11KT were very high (89 ± 5, 92 ± 7, and 91 ± 4%, respectively). Therefore, plasma steroid concentrations reported here are not corrected for extraction efficiency. For all three steroid RIAs, samples of gag plasma diluted parallel to the standard curves over the working ranges used in this study (ANCOVA, $p > 0.05$ for all three steroids). The high specificity of the antisera used in the RIAs was reported previously (Korenman et al. 1974; Hourigan et al. 1991). Conservative upper and lower limits of detection for the

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RIAs (B/B_0 = 20 and 80%) were 0.1 and 2.5 ng/mL, 0.1 and 3.2 ng/mL, and 0.2 and 4.6 ng/mL for E_2, T, and 11KT, respectively.

Seasonal changes in levels of E_2 and T in plasma from female gag followed a similar profile to that observed for plasma VTG levels (Figs. 3B and 3C). Circulating E_2 was nondetectable in plasma from female gag caught during summer and present at very low levels (<0.05 ng/mL) in plasma from fish sampled in fall, just prior to the onset of vitellogenesis. Plasma E_2 levels were intermediate (~0.5 ng/mL) in females captured during the winter quarter and reached peak values (~2.5 ng/mL) in females sampled in spring, when most fish were in stage 4 or 5 of maturation, as judged from gonadal histology. In females, plasma T levels were basal (~0.1 ng/mL) in summer and increased only slightly by fall. They reached intermediate values (~0.4 ng/mL) in winter followed by peak values (~2.2 ng/mL) during the spring quarter. We previously reported that plasma levels of E_2 and T are highly correlated with, and follow the same maturational profile as, VTG in plasma from female gag sampled at different stages of ovarian maturity (Heppell and Sullivan 1999). There was a slight seasonal variation in circulating 11KT in females, with peak levels offset from the spawning period (spring) by one quarter. Maximum plasma 11KT levels (~0.7 ng/mL) were measured in females caught during summer and fall, whereas minimum levels (~0.2 ng/mL) were measured in plasma from females sampled during winter and spring (Fig. 3D). In females, there was a weak but highly significant inverse correlation between circulating 11KT concentrations and plasma levels of VTG ($r = -0.34$, $p << 0.001$, $n = 79$), E_2 ($r = -0.28$, $p << 0.001$, $n = 79$), and T ($r = -0.17$, $p << 0.001$, $n = 79$).

E_2 was detected at a low level (0.1 ng/mL) in plasma from only one out of the four males. Mean (±SEM) springtime levels of T and 11KT in the males were 4.0 ± 0.5 ng/mL and 8.3 ± 0.5 ng/mL, respectively. E_2 was present at a low level (0.1 ng/mL) in plasma from the fish captured while undergoing sex change. Androgen levels in this animal (T, 0.9 ng/mL; 11KT, 2.2 ng/mL) fell well above the range measured in females caught at the same time but only slightly below the range of values measured in mature males. In conjunction with the gonadal histology of this animal (Fig. 2C), these results suggest that this fish was maturing as a functional male at the time of capture.

VTG was present in saline extracts of gag muscle and the extracts diluted parallel to a standard curve in the VTG ELISA (ANCOVA, $p > 0.05$). All three sex steroids were detected in organic extracts of gag muscle. The muscle extracts diluted parallel to E_2, T, and 11KT standards in their respective RIAs (ANCOVA, $p > 0.05$ for all three steroids). The efficiency of steroid extraction from muscle, based on recovery of tritiated E_2, T, or 11KT from triplicate aliquots of muscle homogenate, was 50 ± 7, 55 ± 5, and 59 ± 2%, respectively. Concentrations of steroids in muscle that are reported here are not corrected for extraction efficiency and so should represent about half of the actual steroid levels in gag muscle.

The profiles of seasonal changes in VTG and steroid hormones measured in muscle from female gag were very similar to those seen for plasma levels of these compounds (Figs. 3E–3H). Muscle concentrations of VTG, E_2, and T were nondetectable or low during summer and fall, intermediate in winter, and reached peak values during the springtime spawning season. Muscle levels of VTG in females were highly correlated with plasma VTG levels in the same fish ($r = 0.78$, $p << 0.001$, $n = 63$). Likewise, muscle levels of E_2 and T covaried with plasma levels of these hormones in females (E_2: $r = 0.82$, $p << 0.001$, $n = 54$; T: $r = 0.61$, $p << 0.001$, $n = 57$).

For individual fish, VTG was generally detected in muscle as a constant percentage (2.6 ± 0.3%, $n = 54$) of their plasma VTG levels (Fig. 3A). The same was true for E_2 (16.1 ± 2.9%, $n = 57$) and T (6.3 ± 0.6%, $n = 107$) (Figs. 3B and 3C). However, 11KT was detected in muscle of female gag as a more variable percentage (5.5 ± 1.9%, $n = 35$) of their plasma 11KT levels because the correlation between plasma and muscle 11KT levels was weak, although highly significant ($p << 0.001$) (Fig. 3D). As with the plasma analyses, measurement of 11KT in muscle revealed a weak but significant inverse correlation with muscle levels of VTG ($r = -0.182$, $p < 0.001$), E_2 ($r = -0.066$, $p < 0.001$), and T ($r = -0.149$, $p < 0.001$).

ELISA of VTG appeared to be a promising method for discriminating between male and female gag as well as between mature and immature fish. Of the 41 females identified as mature based on gonadal histology, 35 were judged to be mature by detection of VTG in their muscle. The results can be summarized by stage of ovarian maturation and annual quarter as follows. VTG was detected in muscle from seven of 11 stage 3 fish and both stage 4 fish caught during winter. It was not detected in the one stage 3 fish captured in spring but was detected in all 27 stage 4 or 5 fish caught during this time. No females at ovarian maturity stage 0 or 1 had detectable levels of VTG in their muscle during any quarter ($n = 86$). Out of 17 individuals collected, only one stage 2 female had detectable VTG in her muscle. This fish also had detectable plasma levels of VTG, as did several other stage 2 females. These individuals had oocytes that had developed well past the primary growth stage and may have been on the verge of initiating VTG uptake and processing into yolk proteins for storage in yolk granules, the histological markers of vitellogenic (stages 3 and 4) oocyte growth (Heppell and Sullivan 1999). VTG was not detected in muscle samples from any of the mature males ($n = 4$).

The short-term stability and detectability of VTG and steroids in fresh tissues needs to be established in order for these techniques to be usable in the multiday commercial grouper fishery, where fish are eviscerated immediately upon capture and then stored on ice prior to being landed. To test the stability of VTG, a simulated sampling of landed fish was conducted using white perch (*Morone americana*). Tissues were sampled as described for gag, and VTG was measured immediately upon collection and following storage of tissue at 4°C for 8 days. VTG was detectable at both time points in plasma and muscle from vitellogenic perch, with a small increase in value in both tissues over the 8-day period, but VTG was not detected at either time point in male (control) tissues (Heppell 1998). The reproductive steroids are stable in biological samples for at least 8 weeks at 4°C (Worthman and Stallings 1997).

Size- and age-based maturity schedules (Fig. 4) were also constructed to evaluate the feasibility of judging maturity of

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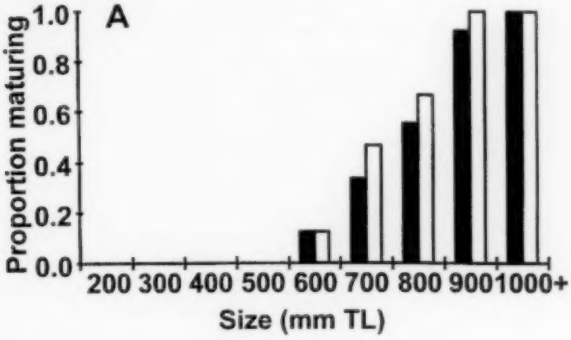
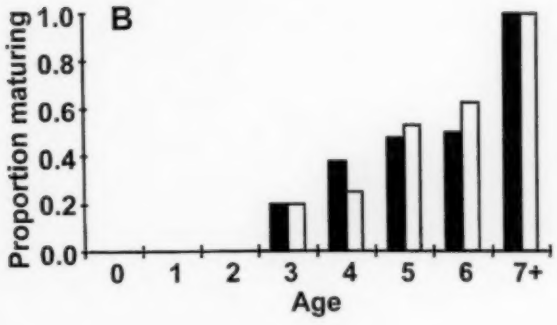
Fig. 4. (A) Size-based and (B) age-based maturity schedules for gag predicted by the presence of VTG in the their muscle versus stage of ovarian maturity verified by routine histological analysis. The proportion of mature females in each size or age (years) category is shown. Females were judged to be mature if their ovary was classified as stage 3, 4, or 5 or if they had detectable levels of VTG in their muscle. Open bars indicate results based on VTG detection and solid bars indicate results based on ovarian histology. The TL categories are indicated by the numbers shown, which identify the maximum length of fish in each size category. Sample sizes for the nine size-classes were n = 8, 15, 9, 3, 4, 11, 27, 13, and 3, respectively. Sample sizes for the eight age categories were n = 6, 19, 26, 5, 16, 19, 8, and 9, respectively.





female gag based on detection of VTG in their muscle. The proportion of mature females in several size (TL) or age categories was evaluated. A maturity schedule based on VTG detection was compared with one based on stage of ovarian maturity. For this comparison, any fish whose ovary was classified as stage 3, 4, or 5 was assumed to be mature, as was any fish with detectable levels of VTG in her muscle. For the female gag, agreement between estimates of maturity based on ovarian stage versus detection of VTG was 100% for the 600 mm TL and lower size-classes and for the 1000 mm TL size-class. For the 700, 800, and 900 mm TL size-classes, the proportion of mature females was slightly underestimated using detection of VTG in muscle in place of ovarian histology as a means of judging maturity. However, the two size-based female maturity schedules did not significantly differ (χ^2 = 1.15, $p < 0.01$ for 4 df). The smallest fish identified as mature were in the 600 mm TL size-class, approximately 50% of females are mature at 700 mm TL, and all females are mature in the 900 and 1000 mm TL size-classes. As noted, VTG was always undetectable in males. Age-based maturity schedules were constructed using the same criteria as above (Fig. 4). As with the size-based maturity schedule, there are some apparent differences, but these were not statistically significant (χ^2 = 1.30, $p < 0.01$ for 4 df).

RIA of 11KT in either plasma or muscle allowed identification of mature males with 100% accuracy during the spring quarter, the only period during which males were collected in this study, as 11KT appeared at significantly higher levels in these males (Mann–Whitney U, $p < 0.01$) (Fig. 5). The maximum blood plasma level of 11KT in any female, mature or immature, during spring was 0.7 ng/mL, whereas the minimum plasma 11KT level observed in males was 7.4 ng/mL. Muscle levels of 11KT showed a similar pattern. The maximum 11KT level measured in muscle from a female was 0.03 ng/g, while the minimum level measured in muscle from a male was 0.2 ng/g.

Levels of E_2 in muscle from immature gag were not correlated with plasma levels of this hormone (r = 0.14, $p > 0.05$, n = 58), while levels of T showed a significant correlation (r = 0.41, $p < 0.01$, n = 58). In males (n = 4), muscle levels of T and E_2 were not statistically correlated with plasma levels of these two hormones (E_2: r = 0.87, $p > 0.05$; T: r = 0.22, $p > 0.05$).

Discussion

The minimum size-at-maturity observed in this study (695 mm TL) is slightly larger than that reported previously for gag (Collins et al. 1987; McGovern et al. 1998), most likely due to the small sample size of this study. The length–weight scaling parameter a calculated from our sample set differs from that reported by Bullock and Smith (1991) (a = 2.68×10^{-8}), which may be accounted for by the fact that their regression was performed on standard length versus TL measurements in our study. The value of b (2.87) for this data set is similar to that of Bullock and Smith (1991) (b = 2.958) and Schirripa and Goodyear (1994) (b = 3.04). All other growth and demographic parameters fall within the range reported by other researchers. Manooch and Haimovici (1978) obtained values of 1290 mm, 0.12154, and –1.127 for L_{inf}, k, and t_0, respectively, for their von Bertalanffy growth parameters, although they determined age using otolith surface and reported a maximum age of only 13 years. The same parameters were estimated as 1122 mm, 0.183, and –0.9939 by Collins et al. (1987), 1190 mm, 0.166, and –0.62 by Hood and Schlieder (1992), and 1283 mm, 0.1246, and –1.7207 by Johnson et al. (1993). Our histological results indicate that gag, like other grouper, are multiple-clutch, group synchronous spawners, as has been previously proposed (Collins et al. 1987). Collectively, these results indicate that the gag sampled in this study are representative of the species as a whole with respect to growth and demographic parameters.

Evidence is accumulating that there has been a drastic shift in both age-at-maturity and sex ratio in gag. Female gag were sexually mature at smaller sizes during 1994–1995 than during 1976–1982 (McGovern et al. 1998). Intense fish-

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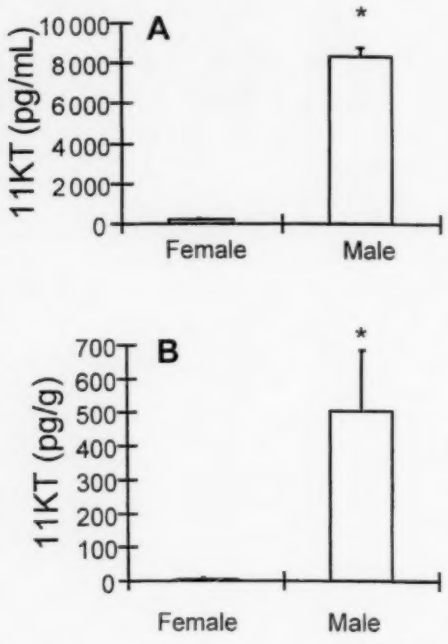
Fig. 5. Spring quarter levels of 11KT in (A) blood plasma and (B) extracts of muscle tissue from male and female gag. Open bars represent the mean value for several fish and vertical brackets indicate SEM. An asterisk indicates a significance difference at $p < 0.05$. Sample sizes were $n = 4$ for males and $n = 60$ for females.



ing pressure is likely responsible for the decrease in female age-at-maturity. Male gag are also clearly disappearing at a rate faster than females can undergo sex change and replace them. Our collection of four males out of 277 fish results in a sex ratio of approximately 70:1. However, this value includes a large number of juvenile animals. When the sex ratio is calculated solely for fish of mature size, which is greater than 600 mm based on fishery data reported by Collins et al. (1987) and Hood and Schlieder (1992), the resulting value is 31:1, similar to that reported by Coleman et al. (1996). This value is much lower than the 8:1 sex ratio observed in the late 1970s and early 1980s by Hood and Schlieder (1992). It has been proposed that the larger, more aggressive males are being selectively removed from the population when fishing occurs on the annual spawning aggregations (Koenig et al. 1996). The effect of this intense fishing pressure may be exacerbated by a downward compression of the gag size–frequency distribution, especially if there is a minimum size threshold below which females are incapable of undergoing sex change. We only captured one fish undergoing sexual transition from female to male in this study. A very low frequency of transitional fish has been typical in prior research on gag and other grouper (Collins et al. 1987; Johnson et al. 1998), possibly because sex change may occur rapidly during or just after the spawning season (McGovern et al. 1998).

Recent documentation of the deleterious changes in gag reproductive biology was possible, in part, due to implementation of an emergency order by the South Atlantic Fishery Management Council arising from concern that gag stocks may be subject to unbearable fishing pressure (South Atlantic Fishery Management Council 1993). This order required certain grouper fishers to land gag intact during 1995 so that histological analysis of their gonads could be performed (McGovern et al. 1998). As noted, this measure is controversial. It adversely impacts the commercial fishers, who normally eviscerate the fish at capture, by preventing them from optimally utilizing ice and space on their vessels. Furthermore, stock assessments that rely on histological processing of gonads are laborious and expensive, require highly skilled personnel to evaluate fish maturity, and are generally not amenable to rapid analyses of a large number of samples. The present study was undertaken to evaluate whether practical biochemical tests for maturity could be developed based on immunoassays for sex-specific hormones or proteins present in gag muscle. Muscle was a logical choice for these analyses because it would be available in the form of eviscerated fish or fillets for fishery-dependent assessment of the reproductive health of gag stocks.

The steroid RIAs used in this study were previously validated for use with plasma from numerous species of perciform teleosts, including striped bass (*Morone saxatilis*) (Woods and Sullivan 1993), white perch (Jackson and Sullivan 1995), bluegill (*Lepomis macrochirus*) (W. King V and C.V. Sullivan, North Carolina State University, Raleigh, N.C., unpublished), and belted sandfish (*Serranus subligarius*) (Oliver 1995). We validated the same assays for use with gag plasma and muscle. Recovery of the different steroids from muscle is close to that reported by Feist et al. (1990) for extraction of the same steroids from whole larval and juvenile coho salmon (*Oncorhynchus kisutch*).

The egg-yolk precursor protein VTG was targeted for analysis because it is relatively easy to purify and measure by ELISA, completely specific to maturing females, and present in the circulation at very high levels during the main phase of oocyte growth in most fishes (Specker and Sullivan 1994). In prior research, we verified these characteristics for gag VTG and demonstrated that detection of this protein in the circulation could substitute for histological examination of the ovary as a means for identifying maturing females (Heppell and Sullivan 1999). Specifically, we found that VTG was always detectable by ELISA in the blood plasma of females whose largest oocytes were in vitellogenic growth or later stages of maturation (ovarian stage > 3), almost never detected in females at earlier maturational stages, and not detected in males. Changes in circulating levels of E_2, which induces hepatic vitellogenesis in fishes (Mommsen and Walsh 1988), were evaluated in the same females, as were plasma levels of T, a substrate for ovarian synthesis of E_2 in teleosts (Kagawa et al. 1982; Nagahama 1987; Adachi et al. 1990). We utilized steroid RIAs to verify that levels of E_2 and T in plasma from maturing females were well correlated with circulating VTG levels, following the same profile of change over the course of ovarian maturation (Heppell and Sullivan 1999). The steroid RIAs and VTG ELISA were highly sensitive, detecting E_2, T, and VTG in muscle and plasma at levels two to three orders of magnitude lower than physiological levels of these compounds in maturing fish. We reasoned that these immuno-

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assays might be applicable to detect the sex hormones and protein in residual blood remaining in muscle from gag that had been eviscerated, or even in gag fillets.

We also targeted 11KT for analysis in gag plasma and muscle. This androgen is implicated in the control of spermatogenesis and development of secondary sexual characteristics in fishes (Fostier et al. 1983; Koldras et al. 1990; Miura et al. 1991; Borg 1994). It is also implicated in regulation of male reproductive behavior. Plasma 11KT levels are positively correlated with male courtship activity in several species, including some hermaphrodites, that exhibit alternative male phenotypes (Kindler et al. 1989; Cardwell and Liley 1991; Hourigan et al. 1991; Brantley et al. 1993; Oliver 1995). Circulating 11KT concentrations are also known to be elevated during spermiogenesis in a number of marine perciforms (Pankhurst and Carragher 1991), including red grouper (*Epinephelus morio*) (Johnson et al. 1998). Furthermore, stoplight parrotfish (*Sparisoma viride*) undergoing sex change exhibit elevated plasma 11KT levels, as do maturing males, while this androgen is not detectable in the circulation of maturing females (Cardwell and Liley 1991). We previously utilized a highly specific RIA to measure 11KT in the plasma of male temperate basses (genus *Morone*), providing evidence for its involvement in control of gametogenesis in these species (Sullivan et al. 1997). In the present study, this RIA was applied to measure 11KT in gag muscle as a potential test for male gender and maturity.

The seasonal cycles of circulating VTG, T, and E_2 in female gag and their relationship to gonadal maturation are similar to what is seen in other teleosts (Fostier et al. 1983; Tao et al. 1993; Jackson and Sullivan 1995; Johnson et al. 1998). VTG, T, and E_2 levels rise concurrently during the main phase of oocyte growth (vitellogenesis), with peak VTG and steroid levels seen in females at stage 4 of ovarian maturation, just prior to the time of spawning (Heppell and Sullivan 1999). The peak in sex steroid and VTG levels seen in female gag coincides with their attainment of maximum gonadosomatic index (GSI) ((ovary weight/body weight) × 100) as reported by Coleman et al. (1996). Seasonal changes in plasma E_2 and T levels in maturing female gag follow very similar profiles. Levels of the two steroids were highly correlated with one another and also with circulating VTG concentrations, confirming our earlier observations on female gag at different stages of ovarian maturation (Heppell and Sullivan 1999). After the spawning period, blood plasma levels of these compounds decrease to baseline values until gonadal recrudescence resumes in the following year.

We detected immunoreactivity for VTG, E_2, T, and 11KT in muscle extracts from female gag at levels highly correlated with plasma concentrations of these compounds in the same fish and showing the same profile of change with season. VTG and sex steroids were present in gag muscle at relatively constant percentages of their concentrations in blood plasma. These results suggest that the VTG ELISA and steroid RIAs may simply be detecting residual levels of these compounds present in the vasculature perfusing the muscle. Although we report immunoassay results in terms of the nominal concentrations of substances being measured, it should be recognized that the actual identities of the immunoreactive materials in muscle remain to be verified. When VTG enters the ovary, it is rapidly taken up into growing oocytes by receptor-mediated endocytosis and almost immediately cleaved into its constituent yolk proteins (Tao et al. 1993; Specker and Sullivan 1994). Putative receptors for VTG have been detected in muscle of white perch (Tao et al. 1996), but it is unclear whether or not the protein actually enters fish muscle cells to be cleaved, degraded, or utilized therein. In a preliminary study on white perch, we utilized a specific antisera to VTG from this species in Western blotting and in immunocytochemistry experiments on muscle tissue from vitellogenic females, mature males, and immature fish. Only in Western blots of muscle extracts from vitellogenic females was a band corresponding in position to the VTG polypeptide monomer immunostained, and the immunoreactivity in corresponding muscle sections was localized to the vascular compartment and periphery of the muscle cells (S.A. Heppell and C.V. Sullivan, personal observation). These results suggest that VTG in fish muscle may be intact and present mainly in the circulatory system. By virtue of their lipophilic nature, it is possible that the sex steroids detected in muscle extracts could be present as authentic E_2, T, and 11KT or certain metabolites of these parent compounds. The antisera used in the RIAs for these steroids have been documented as having very limited cross-reactivities across wide spectra of structurally related and unrelated steroids (Korenman et al. 1974; Hourigan et al. 1991). As immunoreactivity is present in our samples and the antisera are highly specific, authentic steroid must be present in the muscle tissue.

Although the sample size for males was limited ($n = 4$), it appears that the androgens (T, 11KT) predominate in male plasma and muscle tissue, especially around the time of spawning. The higher circulating and muscle levels of E_2 seen in maturing females and the high levels of 11KT seen in males relative to females suggest that these steroids can be used as biomarkers to identify the gender of adult gag captured in winter and spring. In combination with VTG measurements, this discrimination between the sexes should be definitive, at least near the time and place of spawning. Although detection of VTG in blood plasma from female gag marks the onset and progression of vitellogenic growth or later stages of oocyte maturation in their ovaries (Heppell and Sullivan 1999), and plasma and muscle levels of the protein clearly covary in maturing fish, VTG cannot be detected in plasma or muscle from immature or male gag using our ELISA. Therefore, the presence of VTG in muscle can function as a marker for discriminating between reproductively mature female gag and other fish. When VTG was assayed in conjunction with 11KT, it was easy to distinguish between reproductively mature females, reproductively mature males, and immature animals. However, it appears that neither E_2 nor T levels, as measured in plasma or muscle, can serve as reliable indicators of sex or maturity in gag. Both immature and mature animals had detectable levels of these compounds in both plasma and muscle, with some degree of overlap in values between mature and immature fish as well as between mature males and females.

There was a slight apparent discrepancy between the maturity schedule for female gag based on detection of VTG in muscle extracts versus the schedule based on histological analysis of ovary samples. The two maturity schedules were statistically identical, but there were minor differences be-

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tween them in the percentage of fish that were judged to be mature in the 700, 800, and 900 mm TL size-classes. These differences were all attributable to females at maturational stage 3 (early vitellogenesis). The sensitivity of the ELISA for VTG was such that the protein was not detected in muscle from these fish, which were just beginning to enter vitellogenesis, although it was detected in their blood plasma (Heppell and Sullivan 1999) as well as in plasma and muscle extracts from all females at later stages of ovarian maturation. Estimates of size- or age-at-maturity based on analysis of VTG in muscle samples may be slightly conservative, which could be advantageous for the purposes of fishery management.

Our results for circulating steroid levels in gag corroborate recent reports on a confamilial species, the red grouper (Johnson et al. 1998). Both species are protogynous hermaphrodites with females that are multiple-clutch spawners. Their seasonal profiles of change in circulating E_2, T, and 11KT levels appear to be quite similar. It remains to be verified whether muscle levels of the sex steroids and VTG in red grouper and related species covary with circulating levels of these compound during gametogenesis. If so, concurrent immunoassay of 11KT and VTG may have broad applicability as a test for gender and maturity in grouper. These procedures will still be inherently limited in application to analyses based on fish caught during seasons when they are undergoing active gonadal growth, similar to the practice of constructing maturity schedules for females that are based on detection of vitellogenic oocytes in their ovaries. However, when faced with the problem of managing a fishery that routinely involves evisceration or other processing of the fish at sea, such as the gag fishery, these biochemical assays appear to represent a viable substitute to traditional methods based on histological analyses of the gonads.

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References

Adachi, S., Kajiura, H., Kagawa, H., and Nagahama, Y. 1990. Identification of aromatizable androgens secreted by isolated thecal cell layers from vitellogenic ovarian follicles of a teleost, amago salmon (*Oncorhynchus rhodurus*). Biomed. Res. **11**: 359–363.

Borg, B. 1994. Androgens in teleost fishes. Comp. Biochem. Physiol. **109**: 219–245.

Brantley, R.K., Wingfield, J.C., and Bass, A.H. 1993. Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. Horm. Behav. **27**: 332–347.

Bullock, L.H., and Smith, G.B. 1991. Memoirs of the Hourglass cruises: seabasses (Pisces:Serranidae). Florida Marine Resources Institute, St. Petersburg, Fla.

Cardwell, J.R., and Liley, N.R. 1991. Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae). Horm. Behav. **25**: 1–18.

Coleman, F.C., Koenig, C.C., and Collins, L.A. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fishes, **47**: 129–141.

Collins, M.R., Waltz, C.W., Roumillat, W.A., and Stubbs, D.L. 1987. Contributions to the life history and reproductive biology of gag, *Mycteroperca microlepis* (Serranidae), in the South Atlantic bight. Fish. Bull. U.S. **85**: 648–653.

Feist, G., Schreck, C.B., Fitzpatrick, M.S., and Redding, J.M. 1990. Sex steroid profiles of coho salmon (*Oncorynchus kisutch*) during early development and sexual differentiation. Gen. Comp. Endocrinol. **80**: 299–313.

Fostier, A., Jalabert, B., Billard, R., Breton, B., and Zohar, Y. 1983. The gonadal steroids. *In* Fish physiology. *Edited by* W.S. Hoar, D.J. Randall, and E.M. Donaldson. Vol. 9A. Academic Press, New York. pp. 277–372.

Getz, W.M., and Haight, R.G. 1989. Population harvesting: demographic models of fish, forest and animal resources. Monographs in Population Biology Series. Princeton University Press, Princeton, N.J.

Gilmore, R.G., and Jones, R.S. 1992. Color variation and associated behavior in the Epinepheline groupers, *Mycteroperca microlepis* (Goode and Bean) and *M. phenax* Jordan and Swain. Bull. Mar. Sci. **51**: 83–103.

Gordon, M.R., Owen, T.G., Ternan, T.A., and Hildebrand, L.D. 1984. Measurement of a sex-specific protein in skin mucus of premature coho salmon (*Oncorynchus kisutch*). Aquaculture, **43**: 333–339.

Heppell, S.A. 1998. The reproductive physiology of gag grouper (*Mycteroperca microlepis*). Ph.D. dissertation North Carolina State University, Raleigh, N.C.

Heppell, S.A., and Sullivan, C.V. 1999. Gag (*Mycteroperca microlepis*) vitellogenin: purification, characterization, and use for enzyme-linked immunosorbent assay (ELISA) of female maturity in three species of grouper. Fish Physiol. Biochem. **20**: 361–374.

Hood, P.B., and Schlieder, R.A. 1992. Age, growth, and reproduction of gag, *Mycteroperca microlepis* (Pisces: Serranidae), in the eastern Gulf of Mexico. Bull. Mar. Sci. **51**: 337–352.

Hourigan, T.F., Nakamura, M., Nagahama, Y., Yamauchi, K., and Grau, E.G. 1991. Histology, ultrastructure, and in vitro steroidogenesis of the testes of two male phenotypes of the protogynous fish, *Thalassoma duperrey* (Labridae). Gen. Comp. Endocrinol. **83**: 193–217.

Jackson, L.F., and Sullivan, C.V. 1995. Reproduction of white perch: the annual gametogenic cycle. Trans. Am. Fish. Soc. **124**: 563–577.

Johnson, A.G., Collins, L.A., and Isely, J.J. 1993. Age–size structure of gag, *Mycteroperca microlepis*, from the northeastern Gulf of Mexico. Northeast Gulf Sci. **13**: 59–63.

Johnson, A.K., Thomas, P., and Wilson, R.R. 1998. Seasonal cycles of gonadal development and plasma sex steroid levels in *Epinephelus morio*, a protogynous grouper in the eastern Gulf of Mexico. J. Fish Biol. **52**: 502–518.

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Kagawa, H., Young, G., and Nagahama, Y. 1982. Estradiol-17β production in isolated amago salmon (*Oncorhynchus rhodurus*) ovarian follicles and its stimulation by gonadotropins. Gen. Comp. Endocrinol **47**: 440–448.

Kindler, P.M., Philipp, D.P., Gross, M.R., and Bahr, J.M. 1989. Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus*: Centrarchidae). Gen. Comp. Endocrinol. **75**: 446–453.

Kishida, M., Anderson, T.R., and Specker, J.L. 1992. Induction by estradiol-17β of vitellogenin in striped bass (*Morone saxatilis*): characterization and quantification in plasma and mucus. Gen. Comp. Endocrinol. **88**: 29–39.

Koenig, C.C., Coleman, F.C., Collins, L.A., Sadovy, Y., and Colin, P.L. 1996. Reproduction in gag, *Mycteroperca microlepis* (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. *In* Biology, fisheries, and culture of tropical groupers and snappers. *Edited by* F. Arreguin-Sanchez, J.L. Munro, M.C. Balgus, and D. Pauly. ICLARM Conf. Proc. **48**: 307–323.

Koldras, M., Bieniarz, K., and Kime, D.E. 1990. Sperm production and steroidogenesis in testes of the common carp, *Cyprinus carpio* L., at different stages of maturation. J. Fish Biol. **37**: 635–645.

Korenman, S.G., Stevens, R.H., Carpenter, L.A., Robb, M., Niswender, G.D., and Sherman, B.M. 1974. Estradiol radioimmunoassay without chromatography: procedure, validation, and normal values. J. Clin. Endocrinol. Metab. **38**: 718–720.

Manooch, C.S., and Haimovici, M. 1978. Age and growth of the gag, *Mycteroperca microlepis*, and size–age composition of the recreational catch off the southeastern United States. Trans. Am. Fish. Soc. **107**: 234–240.

McGovern, J.C., Wyanski, D.M., Pashuk, O., Manooch, C.S., III, and Sedberry, G.R. 1998. Changes in the sex ratio and size at maturity of gag, *Mycteroperca microlepis*, from the Atlantic coast of the southeastern United States during 1976–1995. Fish. Bull. U.S. **98**: 797–807.

Miura, T., Yamauchi, K., Takahashi, H., and Nagahama, Y. 1991. Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel (*Anguilla japonica*). Proc. Natl. Acad. Sci. U.S.A. **88**: 5774–5778.

Mommsen, T.P., and Walsh, P.J. 1988. Vitellogenesis and oocyte assembly. *In* Fish physiology. Vol. 11A. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 347–406.

Nagahama, Y. 1987. Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. Zool. Sci. **4**: 209–222.

Oliver, A.S. 1995. Hermaphroditism in *Serranus subligarius* (Serranidae: Perciformes): social interactions, size, and sex steroids define a mating system. Ph.D. dissertation, Duke University, Durham, N.C.

Pankhurst, N.W., and Carragher, J.F. 1991. Seasonal endocrine cycles in marine teleosts. *In* Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish. *Edited by* A.P. Scott, J.P. Sumpter, D.E. Kime, and M.S. Rolfe. FishSymp 91, Sheffield, U.K. pp. 131–135.

Pitcher, T.J., and Hart, P.J.B. 1982. Fisheries ecology. Croom Helm, Ltd., London, U.K.

Schirripa, M.J., and Goodyear, C.P. 1994. Status of the gag stocks of the Gulf of Mexico: Assessment 1.0. Contribution MIA-93/94-61, National Marine Fisheries Service, Southeast Fisheries Center, Miami Laboratory, Miami, Fla.

South Atlantic Fishery Management Council. 1993. Amendment number 6, regulation impact review: initial regulatory flexibility analysis and environmental assessment for the snapper/grouper fishery of the south Atlantic region. South Atlantic Fishery Management Council, 1 Southpark Circle, Suite 306, Charleston, SC 29407-4699, U.S.A.

Specker, J.L., and Anderson, T.R. 1994. Developing an ELISA for a model protein — vitellogenin. *In* Biochemistry and molecular biology of fishes. Vol. 3. Analytical techniques. *Edited by* P.W. Hochachka and T.P. Mommsen. Elsevier Biomedical Publishers, New York. pp. 567–578.

Specker, J.L., and Sullivan, C.V. 1994. Vitellogenesis in fishes: status and perspectives. *In* Perspectives in endocrinology. *Edited by* K.G. Davey, R.E. Peter, and S.S. Tobe. National Research Council, Ottawa, Ont. pp. 304–315.

Sullivan, C.V., Berlinsky, D.L., and Hodson, R.G. 1997. Reproduction. *In* Striped bass and other *Morone* culture. Developments in aquaculture and fisheries science. Vol. 30. *Edited by* R.M. Harrell. Elsevier Science B.V., Amsterdam, The Netherlands. pp. 11–73.

Tao, Y., Hara, A., Hodson, R.G., and Sullivan, C.V. 1993. Purification, characterization and immunoassay of striped bass (*Morone saxatilis*) vitellogenin. Fish Physiol. Biochem. **12**: 31–46.

Tao, Y., Berlinsky, D.L., and Sullivan, C.V. 1996. Characterization of a vitellogenin receptor in white perch (*Morone americana*). Biol. Reprod. **5**: 646–656.

Woods, L.C., III, and Sullivan, C.V. 1993. Reproduction of striped bass (*Morone saxatilis*) broodstock: monitoring maturation and hormonal induction of spawning. J. Aquacult. Fish. Manage. **24**: 213–224.

Worthman, C.M., and Stallings, J.F. 1997. Hormone measures in finger-prick blood spot samples: new field methods for reproductive endocrinology. Am. J. Phys. Anthropol. **104**: 1–21.

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Using nonlinear functional relationship regression to fit fisheries models

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Abstract: Functional relationship regression refers to that class of statistical model where a functional relationship is assumed to exist between two arithmetic variables, but the two arithmetic variables can only be viewed with measurement error and (or) natural variability. The goal is to estimate the underlying functional relationship when observing only the variables containing error or natural variability. While statistical details need to be worked out, some general approaches to this class of problem can be recommended. First of all, nonlinear least squares can provide maximum likelihood estimates when the error variance ratio, λ, is assumed known. Furthermore, the usual estimates of standard errors from nonlinear least squares, while theoretically flawed for this class of model, appear to provide usable estimates for many practical problems. Next, a K-sample F test, for testing the equality of nonlinear functional relationship regression curves, is proposed. Finally, computer memory-saving algorithms are suggested for situations where sample sizes are large. Methods proposed here are applied to three types of functional curves commonly estimated in fisheries biology: a stock–recruitment curve, allometry, and the von Bertalanffy growth curve.

Résumé : Les modèles de régression de relation fonctionnelle forment une classe de modèles statistiques pour lesquels on suppose l'existence d'une relation fonctionnelle entre deux variables arithmétiques qui ne peuvent être décelées que par l'erreur de mesure et (ou) la variabilité naturelle. L'objectif recherché consiste à estimer la relation fonctionnelle sous-jacente de par la seule observation des variables présentant une erreur ou de la variabilité naturelle. Bien qu'il reste certains détails statistiques à régler, certaines approches générales à cette classe de problèmes peuvent cependant être recommandées. Tout d'abord, une méthode par moindres carrés non linéaires peut permettre d'obtenir des destinations par maximums de vraisemblance lorsque le rapport de la variance de l'erreur, λ, est supposé connu. En outre, les estimations habituelles des erreurs-types par méthode des moindres carrés non linéaires, qui en théorie s'avèrent biaisées pour cette classe de modèles, semblent cependant fournir des estimations utiles pour la solution de bon nombre de problèmes pratiques. On propose ensuite l'utilisation du test F à échantillon K pour le test de l'égalité des courbes de régression des relations fonctionnelles non linéaires. Pour terminer, des algorithmes économiseurs de mémoire sont proposés pour les situations où l'effectif de l'échantillon est important. Les méthodes proposées sont appliquées à trois types de courbes fonctionnelles couramment estimées en biologie des pêches : la courbe de stock–recrutement, la courbe d'allométrie et la courbe de croissance de von Bertalanffy.

[Traduit par la Rédaction]

Introduction

Ordinary regression generally assumes either that sampling is from a multivariate normal distribution or that independent variables are measured without error and all error is a result of observing the dependent variable. This is not a satisfactory paradigm for many biological problems where nearly all variables are observed with large amounts of either sampling (i.e., measurement) error or natural (i.e., individual genetic and environmental) variability (Ricker 1973). Strictly speaking, these data often fail the assumptions of ordinary regression and can be viewed as requiring measurement error models for proper interpretation. Hilborn and Walters (1992) recognized this "errors in variables" problem and suggested evaluating statistical bias using simulation. Fuller (1987) provided what may be the most definitive treatment of measurement error models in the literature.

There are two basic classes of measurement error models: functional and structural regression. These models are distinguished in their assumptions concerning the "true" independent variable x_i. Functional regression assumes that the true independent variables are unknown constants, while the structural models assume that the true independent variables are normally distributed. The assumptions required for parameter estimation for the linear function are quite different for the two classes of models (e.g., see Kendall and Stuart 1973).

Measurement error models, unfortunately, have had a controversial past that has beclouded their innate simplicity and usefulness. The purpose of this paper is not to contribute further to this debate (the author has already made one attempt; Kimura 1992), but simply to illustrate some statistical methods that are available for the functional relationship regression model. The author believes that functional relationship regression can be a useful alternative to ordinary regression, especially when it is obvious that ordinary regression assumptions do not hold. Towards this end, the author suggests methods for performing the basic tasks of

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estimating parameters, estimating standard errors, and testing the hypothesis of equality between regression curves estimated from different samples. Each of these tasks can be performed reasonably using standard least squares methods with fairly minor adjustments.

Materials and methods

Estimating parameters

Let two mathematical variables $\{x_i,y_i\}$, $i = 1,..., n$, be related through some functional relationship $y_i = f(x_i,\beta)$, where x_i and y_i are scalar but β may be a vector of p variables. We are not able to observe x_i and y_i directly but instead observe the random variables $X_i = x_i + \delta_i$ and $Y_i = y_i + \varepsilon_i$, where δ_i and ε_i are independent normally distributed random variables with mean zero and variances σ_δ^2 and σ_ε^2, respectively. The minus log likelihood for estimating the $n + p$ unknowns $\{\beta_1,..., \beta_p,x_1,..., x_n\}$ is then (Fuller 1987; Seber and Wild 1989):

$$(1) \qquad -L = \sum_{i=1}^{n}\left\{[Y_i - f(x_i,\beta)]^2/(2\sigma_\varepsilon^2) + [X_i - x_i]^2/(2\sigma_\delta^2)\right\} + \text{constant}.$$

Because σ_ε^2 and σ_δ^2 are usually unknown, it is often assumed that the ratio $\lambda = \sigma_\varepsilon^2/\sigma_\delta^2$ is known, and maximum likelihood estimates can be estimated iteratively by minimizing

$$(2) \qquad -2\sigma_\varepsilon^2 L = \sum_{i=1}^{n}\left\{[Y_i - f(x_i,\beta)]^2 + \lambda[X_i - x_i]^2\right\} + \text{constant}.$$

It is important to understand that σ_ε^2 and σ_δ^2 are variances of individual observations around some unknown true values.

If the functional relationship is the simple line $y_i = f(x_i,\beta) = \beta_0 + \beta_1 x_i$, then parameter estimates from least squares will be identical to the usual maximum likelihood parameter estimates given for functional relationship regression coefficients (Kendall and Stuart 1973):

$$(3) \qquad \hat{\beta}_1 = \left\{(s_y^2 - \lambda s_x^2) + [(s_y^2 - \lambda s_x^2)^2 + 4\lambda s_{xy}^2]^{0.5}\right\}/(2s_{xy})$$

$$(4) \qquad \hat{\beta}_0 = \overline{Y} - \overline{X}\hat{\beta}_1$$

where s_x^2, s_y^2, and s_{xy} are the sample variances and covariance of $\{X_i,Y_i\}$ and $\overline{X}$ and $\overline{Y}$ are the sample means.

At this point, we need to contrast the functional relationship method, which is parameter estimates given by eq. 2 for any functional relationship f, with the functional relationships estimated for the simple line given by eqs. 3 and 4. The simple line is, of course, only one type of functional relationship that might be fit.

However, estimation of the simple line has been at the center of controversy of how measurement error models should be used. The problem boils down to the fact that λ will generally not be known. If we assume $\lambda = 1$ in eq. 3, we have the fit called the "major axis." If we assume $\lambda = s_y^2/s_x^2$ in eq. 3, we have the fit called the "standard (or "reduced") major axis." For an interesting discussion concerning the controversy over which of these axes is most generally appropriate, see Jolicoeur (1975, 1990) and Sprent and Dolby (1980). The present author concluded that for functional relationship model fits, a constant value for λ must be assumed (Kimura 1992). Its helpful to understand that if λ is specified, there is little controversy that parameters should be estimated using eq. 2.

Estimating standard errors

Because parameters can be estimated using least squares, the standard error of parameter estimates can be estimated using the gradient matrix of the parameter estimates. Let g be the $2n$-dimensional vector of expected values, $g' = \{f(x_1, \beta),..., f(x_n, \beta), x_1\sqrt{\lambda},..., x_n\sqrt{\lambda}\}$. The $2nx(n + p)$ gradient matrix $Z = \{z_{ij}\}$, where $z_{ij} = \partial g_i/\partial\theta_j$ and $\theta' = \{\beta_1,...,\beta_p,x_1,...,x_n\}$, g_i is the ith entry of g, and θ_j is the jth entry of θ. Also, let $\hat{s}^2 = \sum_{i=1}^{n}\{[Y_i - f(\hat{x}_i,\hat{\beta})]^2 + \lambda[X_i - \hat{x}_i]^2\}/(n - p)$, all evaluated at the least squares solution $\hat{\theta} = \{\hat{\beta}_1,..., \hat{\beta}_p,\hat{x}_1,..., \hat{x}_n\}$. Then the covariance matrix of $\hat{\theta}$ can be estimated as

$$(5) \qquad S(\hat{\theta}) = \hat{s}^2(Z'Z)^{-1}.$$

In many cases, these usual estimates of the covariance matrix from least squares theory can provide usable, approximate estimates of standard errors. A great advantage of using the least squares estimates of standard errors is this easy generalization to any nonlinear functional relationship model.

However, such standard error estimates are technically flawed because they are not statistically consistent. For the simple linear functional relationship, consistent estimates of standard errors are available (Patefield 1977; Fuller 1987). Simulation studies (Kimura 1992) show that for the simple linear model, under ideal conditions, the least squares estimates of standard errors are usually very similar to those provided by Patefield's (1977) consistent estimates. In an "allometry" example, I compare the least squares and Patefield (1977) estimates of standard error.

Although the least squares estimates are convenient, more critical applications may benefit from bootstrap or simulation estimates of standard errors. The simulations may take the form of generic studies of particular models. For example, error variances can be assumed, samples generated, and the least squares standard errors compared with the between-sample standard error of parameter estimates. Bootstrap analysis may be based on residuals of actual model fits.

The least squares method was used to provide the standard errors for the models analyzed in this paper.

Testing the equality of functional relationships in K samples

A final practical problem that I wish to address is that of testing the equality of functional relationships found in each of K samples. Let k denote the sample subscript, so that we now have K functional relationships that we wish to compare: $y_{ki} = f(x_{ki},\beta_k)$. Because parameters were estimated with functional relationship regression (i.e., least squares), it would be natural to test the hypothesis H_0: $\beta_1 = ... = \beta_K$, where the β_ks may be p-dimensional vectors, with the residuals from the functional regression least squares fits. Using the "extra sum of squares" principle, we can calculate an F statistic just as in ordinary regression, but using the functional regression residual sum of squares:

$$(6) \qquad \mathrm{SS}(k) = \sum_{i=1}^{n_k}\{[Y_{ki} - f(\hat{x}_{ki},\hat{\beta}_k)]^2 + \lambda[X_{ki} - \hat{x}_{ki}]^2\}$$

with $n_k - p$ degrees of freedom. However, simulations with the simple linear model clearly demonstrated (Appendix) that under the null hypothesis of equal regression lines, the resulting F statistic, say $\hat{F}_1$, did not always have the expected F distribution.

I therefore tried an alternative F statistic by first combining all K samples, assuming a value for λ, and then performing a functional regression fit estimating $\{\hat{\beta}, \hat{x}_{11},..., \hat{x}_{1n_1}, \hat{x}_{21},..., \hat{x}_{2n_2},..., \hat{x}_{K1},..., \hat{x}_{Kn_K}\}$. This is the usual functional regression model fit under the null hypothesis. Note that for the simple linear model, both $\hat{\beta}$ and the $\{\hat{x}_{ki}\}$ can be estimated in closed form (Appendix). Conditioning on these estimated $\{\hat{x}_{ki}\}$ (i.e., pretending that these are the usual fixed x values for ordinary nonlinear regression), calculate ordinary re-

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Table 1. Functional relationship fits of the eastern Bering Sea walleye pollock stock–recruitment data to the Ricker stock–recruitment curve, $y_i = \beta_0 x_i \exp(-\beta_1 x_i)$, for varying values of λ.

λ	$\hat{\beta}_0$	$\hat{\beta}_1$	SE $\hat{\beta}_0$	SE $\hat{\beta}_1$
10 000	2.7829	0.1356	0.834	0.304
2.0	3.1962	0.1496	0.990	0.033
1.6	3.8228	0.1686	1.242	0.035
1.5	18.7700	0.3425	13.544	0.081
1.4	21.0450	0.3544	16.902	0.088
1.3	24.8227	0.3714	23.091	0.101

Note: Stock–recruitment data and model fits are plotted in Fig. 1.

gression coefficients $\{b_k\}$ and the residual sum of squares (SSR) under the null and alternative hypotheses:

$$\mathrm{SSR}(H_0) = \sum_{k,i} [Y_{ki} - f(\hat{x}_{ki}, \hat{b})]^2$$

$$\mathrm{SSR}(H_A) = \sum_{k,i} [Y_{ki} - f(\hat{x}_{ki}, \hat{b}_k)]^2 .$$

For the combined sample, the functional regression coefficients and the conditional ordinary regression coefficients are identical (i.e., $\hat{b} = \hat{\beta}$) because in this case, the least squares expressions to be minimized are identical for functional and conditional ordinary regressions. However, for the K individual samples, the estimated functional and conditional ordinary regression coefficients will usually be different.

Defining $n = \Sigma n_k$, the degrees of freedom of $\mathrm{SSR}(H_0)$ and $\mathrm{SSR}(H_A)$ are $n - p$ and $n - Kp$, respectively. The sum of squares "due to hypothesis" (SSH) is then $\mathrm{SSH} = \mathrm{SSR}(H_0) - \mathrm{SSR}(H_A)$ with $p(K - 1)$ degrees of freedom. The F statistic calculated from the residuals of conditional ordinary regressions is then

$$\hat{F}_c = \frac{\mathrm{SSH}/[p(K-1)]}{\mathrm{SSR}(H_A)/[n - Kp]} .$$

Simulations (Appendix) show that for the simple linear model, the F statistic based on this conditional ordinary regression F statistic, $\hat{F}_c$, has the central F distribution with $df_1 = p(K - 1)$ and $df_2 = n - Kp$ under the null hypothesis. Because the nonlinear F statistic has been generally useful for testing nonlinear functions using ordinary regression, and because we can show that $\hat{F}_c$ is useful for testing simple lines estimated by functional relationship regression, we suggest that $\hat{F}_c$ be generally used to test nonlinear functions estimated by functional relationship regression.

Memory-saving algorithms

Although the methods described above are equally valid for large as well as small sample sizes (i.e., n), large sample sizes present computational difficulties because the number of parameters to be estimated is $n + p$. Therefore, square matrices of size $n + p$ generally need to be calculated by optimization programs. However, when n is large, personal computers will tend to run out of memory. Fortunately, the parameter estimates and their covariance matrix can be calculated by memory-saving methods.

First of all, parameter estimates can be estimated using a memory-saving EM-type algorithm. Notice that the sum of squares

$$(7) \qquad S(\beta, \{x_i\}) = \sum_{i=1}^{n} \{[Y_i - f(x_i, \beta)]^2 + \lambda[X_i - x_i]^2\}$$

can be minimized by the following.

(1) Initially set $\hat{x}_i = X_i$.

(2) Conditioning on $\{\hat{x}_i\}$, estimate $\hat{\beta}$ by minimizing $S(\beta) = \sum_{i=1}^{n} \{[Y_i - f(\hat{x}_i, \beta)]^2\}$, using an interative function minimizing algorithm.

(3) Next, notice that we can reestimate the $\{\hat{x}_i\}$ in eq. 6 *one at a time* by minimizing $S(\hat{x}_i) = (Y_i - f(\hat{x}_i, \hat{\beta}))^2 + \lambda (X_i - \hat{x}_i)^2$ using Newton's method.

(*a*) We wish to solve $h(x_i) = S'(x_i) = 0$.

(*b*) $h(x_i) = -2[Y_i - f(\hat{\beta}, x_i)] f'(\hat{\beta}, x_i) - 2\lambda[X_i - x_i]$.

(*c*) $h'(x_i) = -2[Y_i - f(\hat{\beta}, x_i)] f''(\hat{\beta}, x_i) + 2 f'(\hat{\beta}, x_i)^2 + 2\lambda$.

The Newton algorithm is $x_{new} = x_{old} - h(x_{old})/h'(x_{old})$. Repeat step 3 until all $\{\hat{x}_i\}$ are reestimated. Repeat steps 2 and 3 until $\hat{\beta}$ and $\{\hat{x}_i\}$ have converged. The convergence criterion that we used in our example was proportional changes between iterations of less than 0.00001 for $\hat{x}_i$ and 0.0001 for the components of $\hat{\beta}$.

Similar memory problems arise when estimating the covariance matrix of parameter estimates. Fortunately, memory-saving methods are also easily devised for calculating the covariance matrix estimates of the parameter estimates when using the gradient matrix. As described earlier, once $\hat{\beta}$ has been estimated, the gradient matrix can be described as

$$Z = \{z_{ij}\} = \begin{pmatrix} G & D \\ O & \sqrt{\lambda} I \end{pmatrix}$$

where G is $n \times p$, D is an $n \times n$ diagonal matrix, O is an $n \times p$ matrix of zeros, and $\sqrt{\lambda} I$ is an $n \times n$ diagonal matrix with $\sqrt{\lambda}$ on the diagonal. It follows that

$$A = \begin{pmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{pmatrix} = Z'Z = \begin{pmatrix} G'G & G'D \\ D'G & D'D + \lambda I \end{pmatrix}$$

$$\text{Let } B = A^{-1} = \begin{pmatrix} B_{11} & B_{12} \\ B_{21} & B_{22} \end{pmatrix}$$

An elementary result from matrix algebra is that if A is a positive definite symmetric matrix, then $B_{11}^{-1} = A_{11} - A_{12}A_{22}^{-1}A_{21}$. Since the covariance matrix of the parameter estimates is $S(\hat{\beta}) = \hat{s}^2 B_{11}$, it is possible to make this calculation with no matrices larger than $n \times p$. This is because $A_{22} = D'D + \lambda I$ is a diagonal matrix that can be inverted simply by inverting its diagonal elements. Furthermore, premultiplying by a diagonal matrix can be accomplished by simply multiplying each row of the target matrix by its corresponding diagonal element.

Results and discussion

Model applications

We now apply the methods presented in this paper to fit and evaluate three models described by Ricker (1975) and commonly used in fisheries biology: the Ricker stock–recruitment curve, the allometric weight–length relationship, and the von Bertalanffy growth curve. These models were fit to data collected from walleye pollock (*Theragra chalcogramma*) in the Bering Sea and Gulf of Alaska. The Ricker curve was fit to 1964–1994 stock–recruitment data taken from the eastern Bering Sea. The allometric model and von Bertalanffy growth curve were fit to 1995 samples taken from commercial fisheries in the Gulf of Alaska. These

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Fig. 1. Functional relationship fits of eastern Bering Sea walleye pollock stock–recruitment data to the Ricker stock–recruitment curve, $y_i = \beta_0 x_i \exp(-\beta_1 x_i)$, for varying values of λ. Parameter estimates and standard errors are provided in Table 1.

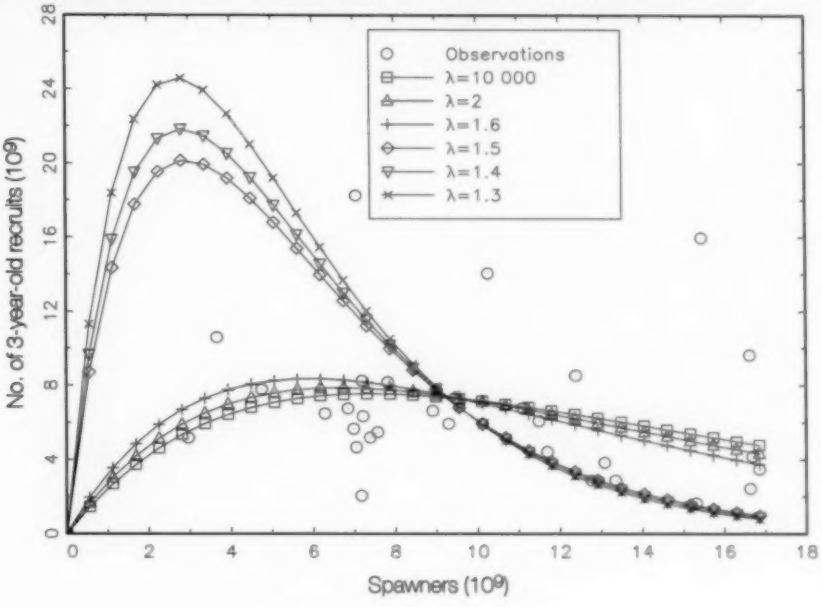

Fig. 2. Overall functional relationship fit of the Gulf of Alaska walleye pollock length–weight data to the allometric curve, $y_i = \beta_0 x_i^{\beta_1}$, where x_i is length and y_i is weight. Following log transformation, the allometric model becomes linear, $\log(y_i) = \log(\beta_0) + \beta_1 \log(x_i) = \beta_0' + \beta_1 \log(x_i)$. Data are plotted indicating age-classes, and length and weight were log transformed and assumed to have equal coefficients of variation so that $\lambda = 1$. Overall functional relationship regression coefficients and regression coefficients by age-class are provided in Table 2.

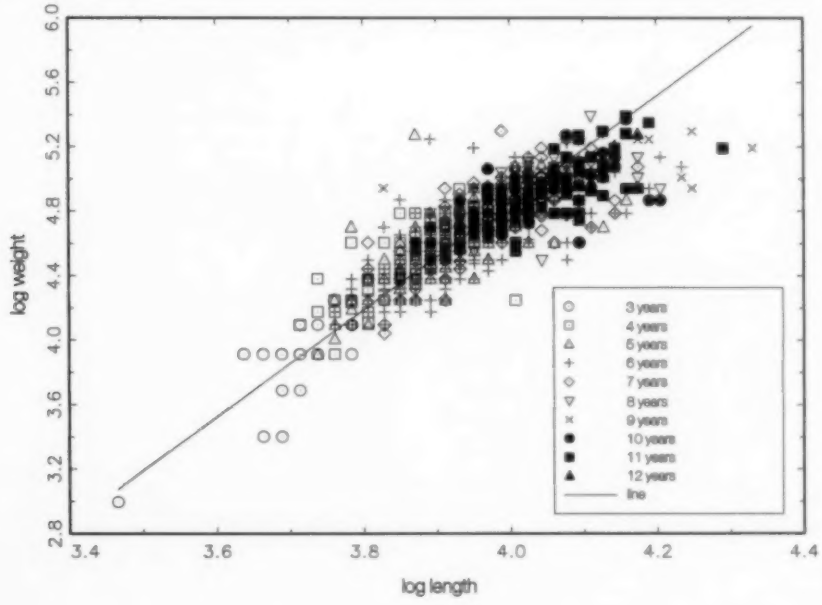

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Table 2. Functional relationship fits of the Gulf of Alaska walleye pollock length–weight data to the allometric curve, $y_i = \beta_0 x_i^{\beta_1}$, where x_i is length and y_i is weight.

Age-class	Sample size	$\hat{\beta}_0'$	$\hat{\beta}_1$	Patefield SE $\hat{\beta}_0'$	Patefield SE $\hat{\beta}_1$	NLS SE $\hat{\beta}_0'$	NLS SE $\hat{\beta}_1$
3	25	–14.795	5.033	2.600	0.698	2.557	0.687
4	86	–10.717	3.933	1.419	0.366	1.364	0.352
5	168	–10.168	3.774	0.975	0.249	0.935	0.239
6	365	–10.241	3.780	0.679	0.173	0.650	0.165
7	197	–10.488	3.838	0.869	0.218	0.838	0.211
8	69	–9.256	3.527	1.721	0.429	1.616	0.403
9	41	–3.497	2.081	1.650	0.408	1.381	0.341
10	44	–8.251	3.257	2.543	0.629	2.284	0.565
11	55	–10.046	3.689	1.990	0.490	1.882	0.463
12	8	–5.235	2.514	2.146	0.528	2.074	0.510
All	1058	–8.450	3.325	0.265	0.067	na	na

Note: Data were separated into age-classes, and length and weight were log transformed and assumed to have equal coefficients of variation so that $\lambda = 1$. Following log transformation, the allometric model becomes linear, $\log(y_i) = \log(\beta_0) + \beta_1 \log(x_i) = \beta_0' + \beta_1 \log(x_i)$. Standard errors were calculated using Patefield's (1977) consistent estimates and the usual nonlinear least squares (NLS) estimates. The log-transformed data and the overall allometric line are plotted in Fig. 2. na, not available.

Table 3. Functional relationship fits of Gulf of Alaska walleye pollock length-at-age data to the von Bertalanffy growth curve, $y_i = \beta_0[1 - \exp(-\beta_1(x_i - \beta_2))]$, for varying values of λ.

λ	$\hat{\beta}_0$	$\hat{\beta}_1$	$\hat{\beta}_2$	SE $\hat{\beta}_0$	SE $\hat{\beta}_1$	SE $\hat{\beta}_2$
10 000	61.097	0.211	–2.920	1.198	0.029	0.745
50	60.794	0.248	–1.725	0.905	0.028	0.517
25	60.948	0.273	–0.966	0.811	0.028	0.449
20	60.869	0.294	–0.522	0.752	0.030	0.419

Note: Methods developed for large sample sizes were used (see text). Age–length data and von Bertalanffy fits are plotted in Fig. 3.

examples were chosen to illustrate different aspects of the methods discussed in this paper.

Ricker stock–recruitment curve

We use the Ricker stock–recruitment curve to illustrate fitting the basic nonlinear functional relationship using eq. 2 to estimate parameters and eq. 5 to estimate standard errors. The Ricker form of the stock–recruitment curve can be described as $y_i = \beta_0 x_i \exp(-\beta_1 x_i)$, where x_i is the number spawning and y_i is the number recruited at age 3 years. Because the number of spawners and number of recruits are both difficult to estimate (Walters and Ludwig 1981), it is natural to suspect that both variables contain a great deal of measurement error. We fit this model to eastern Bering Sea walleye pollock population stock–recruitment data (Table 1), assuming λ = 10 000, 2, 1.6, 1.5, 1.4, and 1.3. Recall that λ = 10 000 is equivalent to ordinary nonlinear least squares, since large values of λ assume that there is relatively little error in the X_i variables. The results (Fig. 1; Table 1) illustrate that for ordinary nonlinear least squares (λ = 10 000) down to functional relationship fits with $\lambda = 1.6$, the model fits differ only slightly, and for $\lambda = 1.5$ down to $\lambda = 1.3$, the model fits are again similar. Around $\lambda = 1.527$ (i.e., when the measurement error of recruitment is 1.527 times the measurement error of stock size), the fit changes dramatically. With $\lambda = 1.5$, the estimated stock–recruitment curve shows much more compensation than the curve estimated using ordinary regression. Possible factors giving credence to this result are that cannibalism is thought to be an important factor in regulating the walleye pollock population and the population has shown considerable stability under significant fishing pressure.

On the other hand, one can argue that there is little direct evidence in the plot (Fig. 1) to support the Ricker curve fit with $\lambda = 1.5$, although this result may be due to the existence of large amounts of error in the data. Perhaps the main conclusion from this analysis should be that we cannot dismiss the possibility of significant compensation in the stock–recruitment relationship.

Alternatively, Quinn and Deriso (1999) applied a linearized form to fit the Ricker stock–recruitment curve using functional relationship regression.

Allometric relationships

Allometric relationships are used by biologists for studying ontogenetic changes and the evolutionary relationship between groups of animals. The allometric model is usually described as $y_i = \beta_0 x_i^{\beta_1}$, where x_i and y_i are two body measurements made on an individual. The data analyst has the choice of fitting the nonlinear relationship directly or mak-

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Fig. 3. Functional relationship fits of Gulf of Alaska walleye pollock length-at-age data to the von Bertalanffy growth curve, $y_i = \beta_0[1 - \exp(-\beta_1(x_i - \beta_2))]$, for varying values of λ. Methods developed for large sample sizes were used (see text). Parameter estimates and standard errors are provided in Table 3.

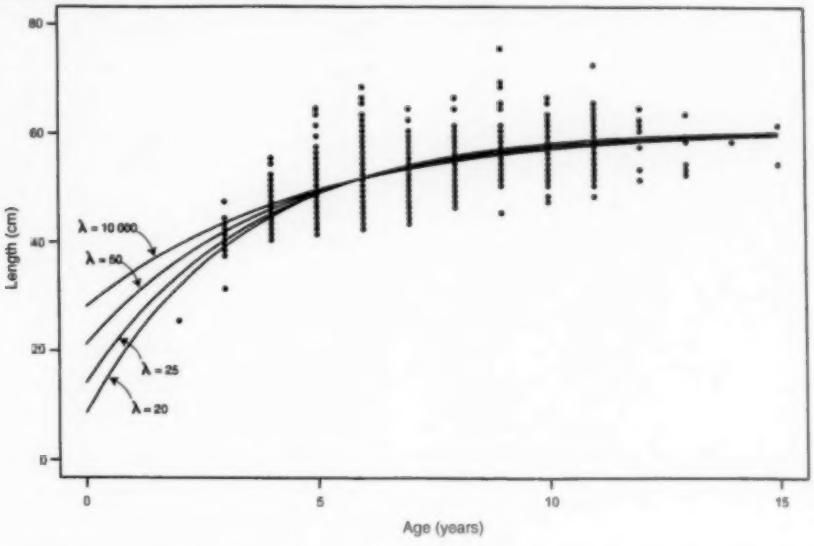
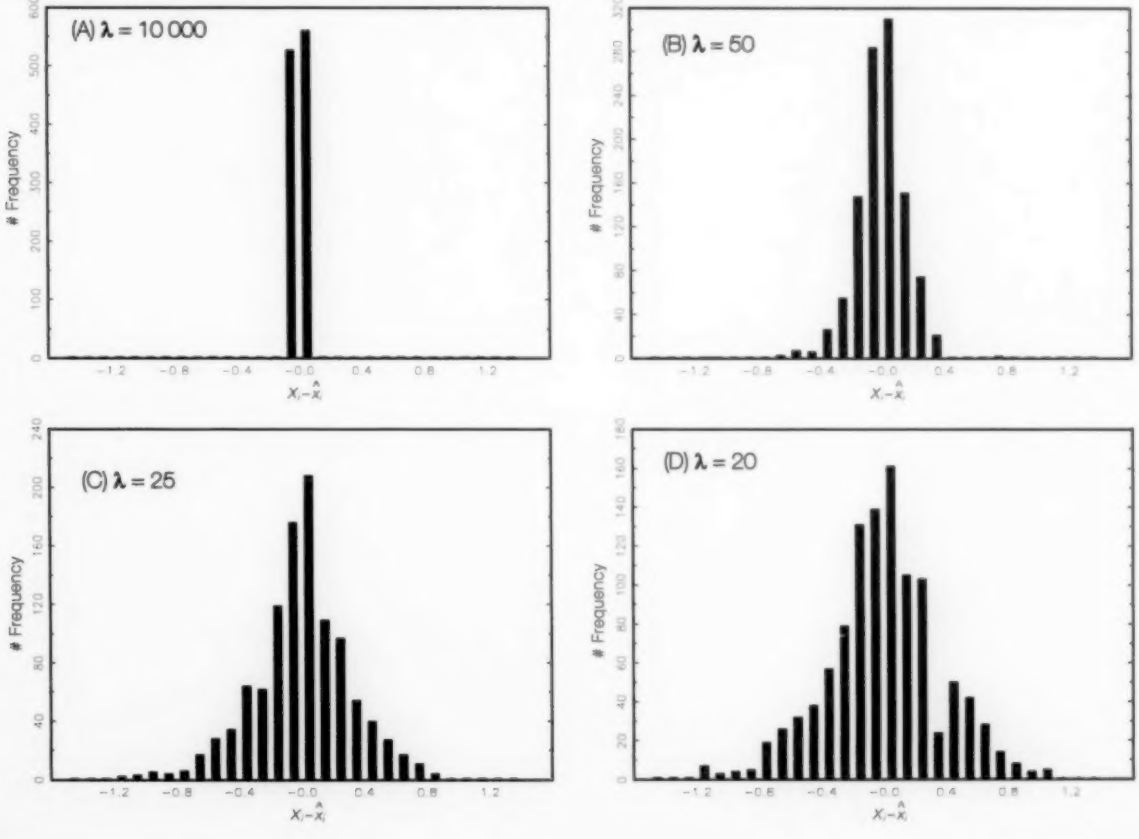


Fig. 4. Histogram of residuals for the X variable, $X_i - \hat{x}_i$, for the functional relationship fits to the von Bertalanffy growth curve for varying values of λ. Regression coefficients are provided in Table 3 and graphed along with data in Fig. 3.



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ing a logarithmic transformation and fitting to a straight line. If logarithms are taken, then $\lambda = CV_y^2/CV_x^2$ where the CVs are the coefficients of variation (i.e., measurement error or natural variability) of individual measurements.

For example, even though length and weight can be accurately determined, obviously the relationship between length and weight will not fall exactly on a curve (i.e., some fish are fat and some are skinny). This is due to what Ricker (1973) called "natural variability," a combination of genetic and environmental factors. One can think of natural variability as imposing measurement error on "true" unobserved variables, so that it appears reasonable to fit a functional relationship regression model.

In contrast, it is common to fit the length–weight relationship simply for the purpose of converting lengths to weights, since measuring lengths of fishes can be much easier than measuring weight. For the purpose of converting length to weight, perhaps ordinary regression is sufficient, since it can be argued that both length and weight can be easily measured with a great deal of accuracy, and one is not concerned about the "true" underlying relationship between length and weight, but only in a mechanism for conversion.

We analyze the length–weight functional relationship for Gulf of Alaska walleye pollock, where x_i is length and y_i is weight, using the log-transformed data with $\lambda = 1$. This means that we assume that the coefficient of variation of individual length and weight observations is the same. Because the logarithmic transformation of the allometric model is a simple linear model, $\log(y_i) = \log(\beta_0) + \beta_1 \log(x_i) = \beta_0' + \beta_1 \log(x_i)$, the data can be fit using closed-form solutions eqs. 3 and 4. For the simple linear model $y_i = \beta_0 + \beta_1 x_i$, the "true" $\{x_i\}$ can be estimated using $\hat{x}_i = [X_i + (Y_i\hat{\beta}_1 - \hat{\beta}_0\hat{\beta}_1)/\lambda]/[1 + \hat{\beta}_1^2/\lambda]$ (Appendix). The question we ask in this analysis is whether the length–weight relationship varies by age. The data were separated into 10 age groups, ages 3–12 years (Fig. 2). The log-transformed data were first fit with all of the age groups combined (Fig. 2). The hypothesis test described earlier was then applied to the problem of whether the allometric line was the same for the 10 age groups.

The individual functional relationship lines (Table 2) show what appears to be a smaller slope coefficient β_1 for older age fish. This is also evident in the data plots (Fig. 2) where the older age groups (i.e., solid symbols) appear to have a smaller slope than the overall functional relationship line. The test statistic $\hat{F}_c = 6.861$, with $df_h = 18$ and $df_e = 1038$, indicates that the functional lines are significantly different ($P[\hat{F}_c > 6.861] \approx 1.2 \times 10^{-16}$).

In this example, we also compare the least squares estimates of standard error with Patefield's (1977) consistent estimates (Table 2). Although the least squares estimates are somewhat smaller than Patefield's (1977) estimates, bias for both estimates is unknown.

von Bertalanffy growth

Fishery biologists use the von Bertalanffy growth curve almost exclusively to fit length-at-age data to describe the growth of fishes. The most common form of the von Bertalanffy growth curve is $y_i = \beta_0[1 - \exp(-\beta_1(x_i - \beta_2))]$, where β_0 is the asymptotic size, β_1 determines the growth rate, and β_2 is the age at length zero. Because age is difficult to determine in fishes, it is reasonable to consider that the age of fish is known with some measurement error. Also, because the length of each fish of the same "true" age is not identical, we know that length-at-age contains considerable natural variability.

The von Bertalanffy growth curve was fit to length-at-age data collected from walleye pollock in the Gulf of Alaska using $\lambda = 10\,000$, 50, 25, and 20. The large value of λ assumes no measurement error in the ages, while the lesser values assume progressively more ageing error relative to the amount of natural variability in length at age.

As in the previous allometry example, the sample sizes are fairly large (in this case, $n = 1087$). However, in the allometric example, we made a logarithmic transformation so that the model was linear and closed-form parameter estimates were available. In the case of the von Bertalanffy model, we must estimate $(\beta_0, \beta_1, \beta_2)$ and all $\{x_i\}$ simultaneously for a total of $n + p = 1090$ parameters. To accomplish this, the memory-saving algorithms described in this paper were used.

The results (Table 3; Fig. 3) show that as greater ageing error is assumed in the data, larger values of the growth rate β_1 are estimated. This suggests that β_1 may be underestimated by not assuming ageing error. One way that we can evaluate the proper range of λ for the model fit is by examining the residuals $X_i - \hat{x}_i$ for different values of λ. The magnitude of the residual will increase as the assumed value of λ decreases (Fig. 4). If $\lambda = 10\,000$, the residuals are small (Fig. 4A), indicating that the model fit is essentially ordinary regression. If the analyst has evidence that ages are in error ±0.5 year, then $\lambda = 50$ seems attractive; if adjustments of ±1.0 year make sense, then $\lambda = 20$ or 25 might appear more attractive. Evidence concerning the magnitude of ageing error might come from between-reader estimates of ageing error or comparisons of reader ages with known ages.

There is not a great deal of data in the region of the plot where the von Bertalanffy growth curve fits differ (Fig. 3). Nevertheless, this analysis suggests that the seemingly smaller value for $\hat{\beta}_1$ estimated using ordinary nonlinear least squares ($\lambda = 10\,000$) may be due to the presence of ageing error in the data.

Conclusions

I have shown that least squares methods are available for estimating and interpreting results from nonlinear functional relationship regression. These methods are straightforward, intuitive extensions to ordinary least squares. Whenever a researcher is interested in possible changes in results that might occur if measurement error and (or) natural variability exists in both X_i and Y_i, the methods presented here provide easy to apply general methods for exploring these changes. Although the suggested methods appear to perform satisfactorily, advances in statistical theory should eventually provide improved methods for estimating standard errors and testing regression curves. Simulation remains an important tool for evaluating modeling results. For example, simulation can be used to evaluate the validity of the gradient (i.e., least squares) method of estimating standard errors for any particular model.

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References

Fuller, W.A. 1987. Measurement error models. John Wiley & Sons, New York.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment. Chapman and Hall, New York.

Jolicoeur, P. 1975. Linear regressions in fishery research: some comments. J. Fish. Res. Board Can. **32**: 1491–1494.

Jolicoeur, P. 1990. Bivariate allometry: interval estimation of the slopes of ordinary and standardized normal major axes and structural relationship. J. Theor. Biol. **144**: 275–285.

Kendall, M.G., and Stuart, A. 1973. The advanced theory of statistics. Vol. 2. Hafner Publishing Co., New York.

Kimura, D.K. 1992. Symmetry and scale dependence in functional relationship regression. Syst. Biol. **41**: 233–241.

Patefield, W.M. 1977. On the information matrix in the linear functional relationship problem. Appl. Stat. **26**: 69–70.

Quinn, T.J., II, and Deriso, R.B. 1999. Quantitative fishery dynamics. Oxford University Press, New York.

Ricker, W.E. 1973. Linear regression in fishery research. J. Fish. Res. Board Can. **30**: 409–434.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Seber, G.A.F., and Wild, C.J. 1989. Nonlinear regression. John Wiley & Sons, New York.

Sprent, P., and Dolby, G.R. 1980. The geometric mean functional relationship. Biometrics, **36**: 547–550.

Walters, C.J., and Ludwig, D. 1981. Effects of measurement errors on the assessment of stock–recruitment relationships. Can. J. Fish. Aquat. Sci. **38**: 704–710.

Appendix

In the Appendix, we examine the problem of testing the equality of several linear regression lines of the form $y_{ki} = \alpha_k + \beta_k x_{ki}$, $k = 1,..., K$, $i = 1,..., n_k$. The null hypothesis for the equality of regression lines is H_0: $\alpha_1 = ... = \alpha_K$ and $\beta_1 = ... = \beta_K$. Although the functional relationships are defined between "true" values (y_{ki}, x_{ki}), only variables containing error are observable: $X_{ki} = x_{ki} + \delta_{ki}$ and $Y_{ki} = y_{ki} + \varepsilon_{ki}$. We assume that all δ_{ki} and ε_{ki} are independent and normally distributed with variances σ_δ^2 and σ_ε^2, respectively.

Assuming the variance ratio $\lambda = \sigma_\varepsilon^2/\sigma_\delta^2$ is known, the maximum likelihood estimates of $(\alpha_k, \beta_k, x_{ki})$ are well known (e.g., Graybill 1961, eqs. 9.20–9.22; Kendall and Stuart 1973, eqs. 29.51 and 29.52; Fuller 1987, eqs. 1.3.21, 1.3.27, and 1.3.28):

$$\hat{\beta}_k = [(s_{yk}^2 - \lambda s_{xk}^2) + [(s_{yk}^2 - \lambda s_{xk}^2)^2 + 4\lambda s_{xyk}^2]^{0.5}]/(2s_{xyk})$$

$$\hat{\alpha}_k = \bar{Y}_k - \bar{X}_k \hat{\beta}_k$$

$$\hat{x}_{ki} = [X_{ki} + (Y_{ki}\hat{\beta}_k - \hat{\alpha}_k\hat{\beta}_k)/\lambda]/[1 + \hat{\beta}_k^2/\lambda].$$

The quantities $(\bar{X}_k, \bar{Y}_k, s_{xk}^2, s_{yk}^2, s_{xyk})$ are simply the sample means, variances, and covariance of the observed (X_{ki}, Y_{ki}) calculated for the kth sample. Fuller's (1987) results are somewhat more general, allowing for covariance between ε_{ki} and δ_{ki}.

The maximum likelihood estimates for the functional relationship model with λ known can also be estimated by minimizing the residual sum of squares for the model fit over *both* the X and Y variables (Graybill 1961, sect. 9.2.2; Fuller 1987, sect. 1.3.3; Kimura 1992*a*, 1992*b*):

$$\mathrm{SSF}(k) = \sum_i \{(Y_{ki} - \hat{y}_{ki})^2 + \lambda(X_{ki} - \hat{x}_{ki})^2\}.$$

It can be noted that the residual degrees of freedom are the same as for ordinary regression (i.e., $n_k - 2$) because while the number of observations is $2n_k$, the number of parameters in the model increases to $2 + n_k$.

The question we pose is how do we test the hypothesis of equal functional regression lines? Using the analogy of ordinary least squares, it is inviting to calculate an F statistic using the extra sum of squares principle (Draper and Smith 1981), but using the functional regression residual sum of squares described above:

$$\mathrm{SSF}(H_0) = \sum_{k,i} \{[Y_{ki} - (\hat{\alpha} + \hat{\beta}\hat{x}_{ki})]^2 + \lambda[X_{ki} - \hat{x}_{ki}]^2\}$$

$$\mathrm{SSF}(H_A) = \sum_{k,i} \{[Y_{ki} - (\hat{\alpha}_k + \hat{\beta}_k\hat{x}_{ki})]^2 + \lambda[X_{ki} - \hat{x}_{ki}]^2\}.$$

Recall that the minimization of $\mathrm{SSF}(H_0)$ is over $\{\hat{\alpha}, \hat{\beta}, \hat{x}_{11},..., \hat{x}_{1n_1}, \hat{x}_{21},..., \hat{x}_{2n_2},..., \hat{x}_{K1},..., \hat{x}_{Kn_K}\}$ and the minimization of $\mathrm{SSF}(H_A)$ is over $\{\hat{\alpha}_1, \hat{\beta}_1,..., \hat{\alpha}_K, \hat{\beta}_K, \hat{x}_{11},..., \hat{x}_{1n_1}, \hat{x}_{21},..., \hat{x}_{2n_2},..., \hat{x}_{K1},..., \hat{x}_{Kn_K}\}$ so that the estimated $\hat{x}_{ki}$ are different under $\mathrm{SSF}(H_0)$ and $\mathrm{SSF}(H_A)$. Defining $n = \Sigma n_k$, the degrees of freedom of $\mathrm{SSF}(H_0)$ and $\mathrm{SSF}(H_A)$ are $n - 2$ and $n - 2K$, respectively. The sum of squares due to hypothesis is then $\mathrm{SSFH} = \mathrm{SSF}(H_0) - \mathrm{SSF}(H_A)$ with $2(K - 1)$ degrees of freedom. The F statistic calculated from the residuals of functional regressions is then

$$\hat{F}_f = \frac{\mathrm{SSFH}/[2(K-1)]}{\mathrm{SSF}(H_A)/[n-2K]}.$$

However, under the null hypothesis of equal regression lines, the resulting F statistic, $\hat{F}_f$, does not always have a distribution well approximated by the central F distribution.

The reason for this may be that the reestimation of the $\{x_{ki}\}$ under the alternative hypotheses prevents a proper orthogonal decomposition of the sum of squares required for the F distribution. Under the classic derivation of the F statistic (Scheffe 1959, sect. 2.9), the sums of squares are squared norms of projections made onto the column space spanned by the observed X data. Under ordinary regression, these X values are constants.

With this idea in mind, I tried an alternative F statistic. First, combine all of the sample data (i.e., all samples), assume a value for λ, and perform a functional regression fit estimating $\{\hat{\alpha}, \hat{\beta}, \hat{x}_{11},..., \hat{x}_{1n_1}, \hat{x}_{21},..., \hat{x}_{2n_2},..., \hat{x}_{K1},..., \hat{x}_{Kn_K}\}$. This is the usual functional regression model fit under the null hypothesis. Conditioning on these estimated $\{\hat{x}_{ki}\}$ (i.e., pretending that these are the usual fixed x values for ordinary regression), calculate ordinary regression coefficients (a, b) and the residual sum of squares under the null and alternative hypotheses:

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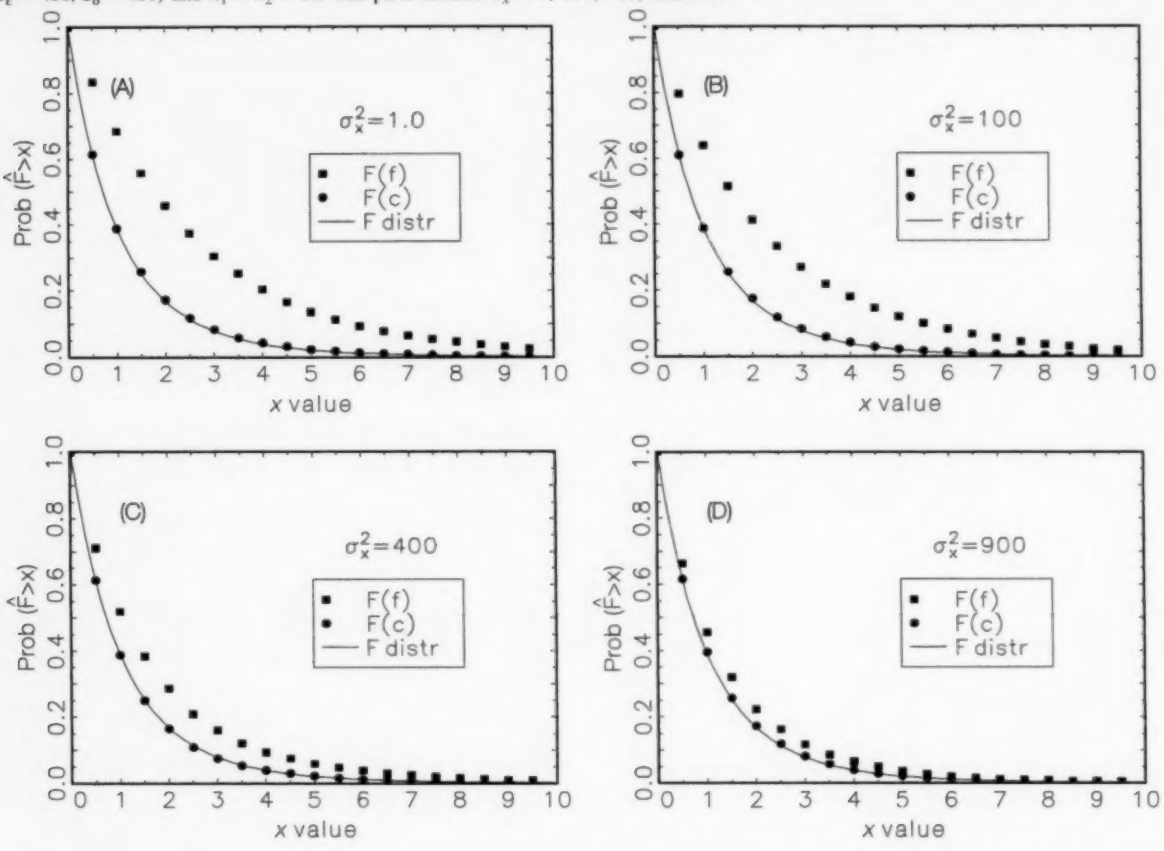
Fig. A1. Comparisons of distribution of simulated F tests, F_f and F_c, under the null hypothesis of equal regression lines for $\lambda = 1.0$ ($\sigma_\varepsilon^2 = 400$, $\sigma_\delta^2 = 400$) and $n_1 = n_2 = 10$. The plots assume $\sigma_x^2 = 1$, 100, 400, and 900.



$$SSC(H_0) = \sum_{k,i} [Y_{ki} - (\hat{a} + \hat{b}\hat{x}_{ki})]^2$$

$$SSC(H_A) = \sum_{k,i} [Y_{ki} - (\hat{a}_k + \hat{b}_k\hat{x}_{ki})]^2.$$

It is intuitively important to note that for the combined sample, the functional regression coefficients and the conditional ordinary regression coefficients are identical (i.e., $\hat{a} = \hat{\alpha}$ and $\hat{b} = \hat{\beta}$). This is because maximum likelihood estimates $\{\hat{\alpha}, \hat{\beta}\}$ can be viewed as the maximization step of the EM algorithm (Kimura 1992*b*), which is precisely this conditional ordinary regression. However, for individual samples, the estimated functional and conditional ordinary regression coefficients will generally be different (i.e., $\hat{a}_k \neq \hat{\alpha}_k$ and $\hat{b}_k \neq \hat{\beta}_k$).

Again, the degrees of freedom of $SSC(H_0)$ and $SSC(H_A)$ are $n - 2$ and $n - 2K$, respectively. The sum of squares due to hypothesis is then $SSCH = SSC(H_0) - SSC(H_A)$ with $2(K - 1)$ degrees of freedom. The F statistic calculated from the residuals of conditional ordinary regressions is then

$$\hat{F}_c = \frac{SSCH/[2(K-1)]}{SSC(H_A)/[n-2K]}.$$

Simulations

We performed a variety of simulations to examine the behavior of $\hat{F}_f$ and $\hat{F}_c$ under different circumstances. All simulations assumed $K = 2$ but varied the following.

(1) $\lambda = \sigma_\varepsilon^2/\sigma_\delta^2$: (*a*) $\lambda = 100$ ($\sigma_\varepsilon^2 = 400$, $\sigma_\delta^2 = 4$), (*b*) $\lambda = 1.0$ ($\sigma_\varepsilon^2 = 400$, $\sigma_\delta^2 = 400$), (*c*) $\lambda = 0.01$ ($\sigma_\varepsilon^2 = 4$, $\sigma_\delta^2 = 400$).

(2) Sample sizes n_k: (*a*) $n_1 = n_2 = 3$, (*b*) $n_1 = n_2 = 10$, (*c*) $n_1 = n_2 = 25$.

(3) $\mathrm{Var}(x_{ij}) = \sigma_x^2$: (*A*) $\sigma_x^2 = 0.1$, (*b*) $\sigma_x^2 = 1.0$, (*c*) $\sigma_x^2 = 100$, (*d*) $\sigma_x^2 = 400$, (*e*) $\sigma_x^2 = 900$, (*f*) $\sigma_x^2 = 10\,000$.

(4) Simulations under the null hypothesis of equal regression lines assumed (*a*) ($\alpha_1 = 0.0$, $\beta_1 = 0.25$; $\alpha_2 = 0.0$, $\beta_2 = 0.25$), (*b*) ($\alpha_1 = 0.0$, $\beta_1 = 1.0$; $\alpha_2 = 0.0$, $\beta_2 = 1.0$), (*c*) ($\alpha_1 = 0.0$, $\beta_1 = 1000$; $\alpha_2 = 0.0$, $\beta_2 = 1000$).

Each simulation was repeated 10 000 times, and measurement errors and true x values were assumed to be normally distributed with a mean of zero. This variability in x values is in accordance with the "structural relationship" model (Kendall and Stuart 1973, sect. 29.6).

Simulation 1

Assumed 1*abc*, 2*b*, 3*bcde*, 4*b*. The purpose of this simulation is to show the null distribution of $\hat{F}_f$ and $\hat{F}_c$ for varying λ and σ_x^2.

Simulation 2

Assumed 1*b*, 2*ac*, 3*af*, 4*ac*. The purpose of this simulation

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Fig. A2. Comparisons of distribution of simulated F tests, F_f and F_c, under the null hypothesis of equal regression lines for $\lambda = 100$ ($\sigma_\varepsilon^2 = 400$, $\sigma_\delta^2 = 4$) and $n_1 = n_2 = 10$. The plots assume $\sigma_x^2 = 1$, 100, 400, and 900. These plots are identical to the plots for $\lambda = 0.01$ ($\sigma_\varepsilon^2 = 4$, $\sigma_\delta^2 = 400$), which implies a symmetry for the distribution of F_f under the null hypothesis.

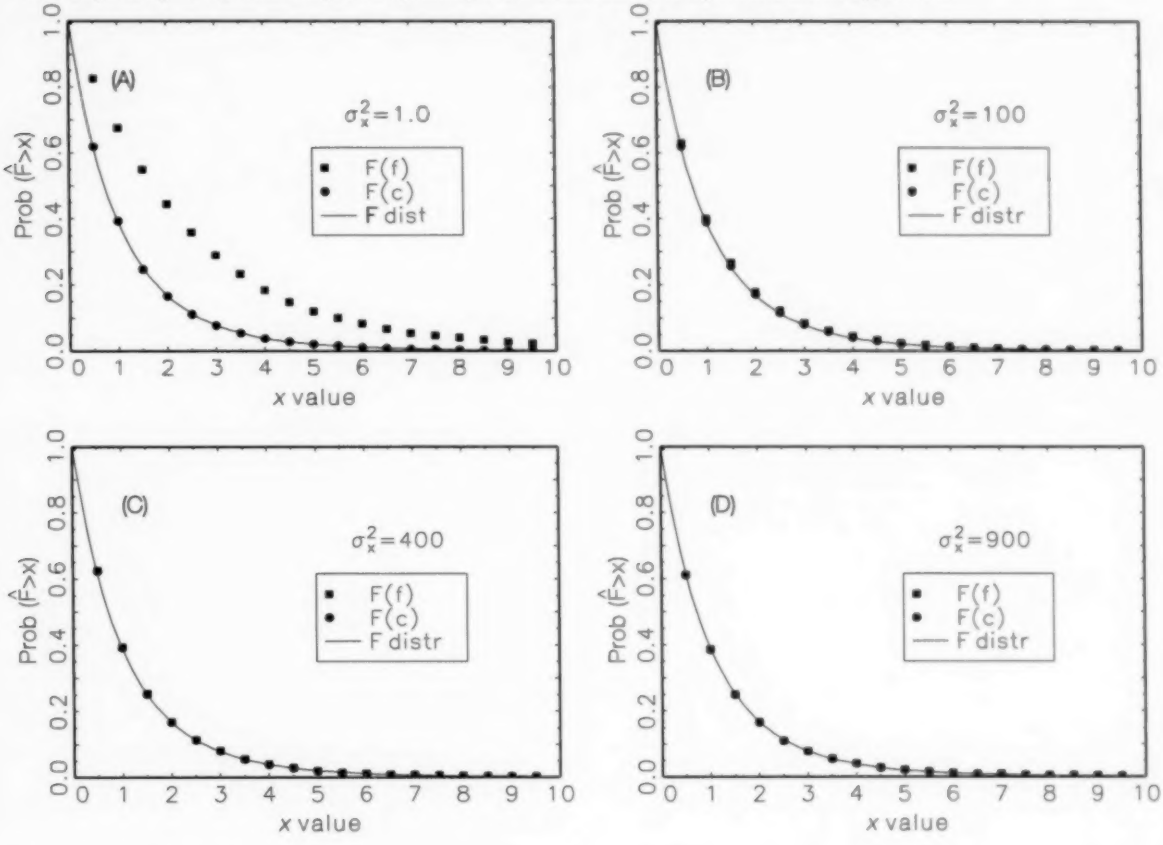

Table A1. Simulated type I error rates (α) for the F_f and F_c statistics compared with values predicted from the central F distribution.

n_1, n_2	σ_ε^2	β	Nominal α	$P[\hat{F}_f > F_{1-\alpha}]$	$P[\hat{F}_c > F_{1-\alpha}]$
3	0.1	0.25	0.10	0.1656	0.1008
			0.05	0.0851	0.0523
			0.01	0.0172	0.0106
3	0.1	1000	0.10	0.0975	0.0951
			0.05	0.0470	0.0458
			0.01	0.0098	0.0097
3	10 000	0.25	0.10	0.1100	0.0987
			0.05	0.0552	0.0487
			0.01	0.0114	0.0101
3	10 000	1000	0.10	0.0960	0.0960
			0.05	0.0478	0.0478
			0.01	0.0101	0.0101

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Table A1 (*concluded*).

n_1, n_2	σ_x^2	β	Nominal α	$P[\hat{F}_f > F_{1-\alpha}]$	$P[\hat{F}_c > F_{1-\alpha}]$
25	0.1	0.25	0.10	0.4669	0.1016
			0.05	0.3607	0.0517
			0.01	0.1851	0.0098
25	0.1	1000	0.10	0.0985	0.0981
			0.05	0.0473	0.0472
			0.01	0.0095	0.0092
25	10 000	0.25	0.10	0.1085	0.1039
			0.05	0.0543	0.0513
			0.01	0.0125	0.0115
25	10 000	1000	0.10	0.1040	0.1040
			0.05	0.0486	0.0486
			0.01	0.0099	0.0099

Note: All simulations assumed $\lambda = 1.0$ ($\sigma_\varepsilon^2 = 400$, $\sigma_\delta^2 = 400$) and were replicated 10 000 times for each value of n_i, σ_x^2, and β.

was to examine the null distribution of $\hat{F}_f$ and $\hat{F}_c$ under a variety of conditions. Generally, I tried to examine two extreme levels of sample size ($n_1 = n_2 = 3$–25), $\sigma_x^2 = 0.1$ – 10 000, and $\beta = 0.25$–1000. These were arranged as a 2^3 factorial design.

Simulation results

Results from simulation 1 (Figs. A1 and A2) indicate that for varying λ and σ_x^2 (i.e., spreads in true x values), the distribution of $\hat{F}_c$ under the null hypothesis of equal regression lines is well approximated by the central F distribution. These simulations also indicate that the null distribution of $\hat{F}_f$ can be poorly approximated (i.e., excessive type I error) by the central F distribution, especially when σ_x^2 is very small (assumption 3*b*). However, when there is substantial measurement error in both X and Y, the poor approximation might occur even if $\sigma_x^2 > \sigma_\varepsilon^2$ and $\sigma_x^2 > \sigma_\delta^2$.

Finally, simulation 2 was designed to explore conditions where the F statistics might fail to have the central F distribution under the null hypothesis. For brevity, I examined simulated type I error rates at the $\alpha = 0.10$, 0.05, and 0.01 levels. Table A1 shows that F_c followed the central F distribution at all levels of the simulation. The F_f also performed quite well except when σ_x^2 and β were both small. Sample size, n_k, did not seem to matter.

From these simulations, we recommend that the hypothesis of equal lines for functional relationships be tested using the F_c statistic. This statistic appears to generally have the correct F distribution even when the spread in x values is very small. Caution should be used when applying the F_f statistic. Under the null hypothesis, simulations show that F_f can have inflated type I error rates even when variability in x exceeds measurement errors. Variability should be evaluated if the F_f statistic is to be used.

Appendix References

Draper, N.R., and Smith, H. 1981. Applied regression analysis. 2nd ed. John Wiley & Sons, New York.

Fuller, W.A. 1987. Measurement error models. John Wiley & Sons, New York.

Graybill, F.A. 1961. An introduction to linear models. Vol. I. McGraw-Hill, New York.

Kendall, M.G., and Stuart, A. 1973. The advanced theory of statistics. Vol. 2. Hafner Publishing Co., New York.

Kimura, D.K. 1992*a*. Symmetry and scale dependence in functional relationship regression. Syst. Biol. **41**: 233–241.

Kimura, D.K. 1992*b*. Functional comparative calibration using an EM algorithm. Biometrics, **48**: 1263–1271.

Scheffe, H. 1959. The analysis of variance. John Wiley & Sons, New York.

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Effects of trophic status and wetland morphology, hydroperiod, and water chemistry on mercury concentrations in fish

Joel W. Snodgrass, Charles H. Jagoe, A. Lawrence Bryan Jr., Heather A. Brant, and J. Burger

Abstract: We sampled fish and selected water chemistry variables (dissolved organic carbon, sulfate, and pH) in nine southeastern depression wetlands to determine relationships among wetland morphology (surface area and maximum depth), hydrology, water chemistry, and bioaccumulation of mercury (Hg) in fishes. We concentrated on three fish species representing the range of trophic levels occupied by fish in southeastern depression wetlands. Whole-body Hg concentrations were lowest in lake chubsucker (*Erimyzon sucetta*), a benthic detritivore, and highest in redfin pickerel (*Esox americanus americanus*), a top carnivore. However, variation in Hg concentrations among wetlands was greater than variation among species. Regression analyses indicated that maximum depth and hydroperiod accounted for significant portions of variation among wetlands in standardized lake chubsucker and redfin pickerel Hg concentrations. Maximum depth and dissolved organic carbon had a negative effect on standardized Hg concentrations in mud sunfish (*Acantharchus pomotis*). Path analysis confirmed the results of regression analyses, with maximum depth and hydroperiod having relatively large direct negative effects on Hg concentrations. Our results suggest that leaching of Hg from sediments during the drying and reflooding cycle and binding of Hg species by dissolved organic carbon in the water column are primary factors controlling the bioavailability of Hg in southeastern depression wetlands.

Résumé : Nous avons prélevé des poissons et analysé certaines variables de la chimie de l'eau (carbone organique dissous, sulfate et pH) dans neuf terres humides de dépressions du sud-est afin de déterminer les rapports entre la morphologie des terres humides (superficie et profondeur maximale), l'hydrologie, la chimie de l'eau et la bioaccumulation du mercure (Hg) chez les poissons. Nous avons concentré nos travaux sur trois espèces de poisson représentatives de la gamme des niveaux trophiques des poissons des terres humides des dépressions du sud-est. Les concentrations de Hg dans tout l'organisme étaient inférieures chez le sucet de lac (*Erimyzon sucetta*), un détritivore benthique, et supérieures dans le brochet d'Amérique (*Esox americanus americanus*), un carnivore de niveau supérieur. La variation des concentrations de Hg était cependant plus importante entre les terres humides qu'entre les espèces. Des analyses de régression ont montré que la profondeur maximum et la période hydrique expliquaient une partie importante de la variation entre les terres humides après utilisation de concentrations standardisées pour le sucet de lac et le brochet d'Amérique. La profondeur maximum et le carbone organique dissous avaient un effet négatif sur les concentrations standardisées de Hg chez *Acantharchus pomotis*. L'analyse des cheminements a confirmé les résultats des analyses de régression, la profondeur maximum et la période hydrique ayant des effets directs négatifs relativement importants sur les concentrations de Hg. Nos résultats indiquent que le lessivage du Hg des sédiments pendant les cycles d'assèchement et d'inondation et la liaison des espèces chimiques du Hg par le carbone organique dissous dans la colonne d'eau constituent les principaux facteurs régissant la biodisponibilité dans les terres humides des dépressions du sud-est.

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Introduction

Bioaccumulation of mercury (Hg) in aquatic food chains represents a risk both to aquatic organisms and to humans and wildlife that feed on aquatic organisms (Clarkson 1990; Thompson 1996; Wiener and Spry 1996). A number of factors specific to individual lake systems have been identified as influencing Hg concentrations in fish, including food web structure, pH, total and dissolved organic carbon (DOC), sulfate levels, and morphometric conditions (Wiener and Spry 1996). Internal factors are often more influencial in controlling accumulation of Hg by aquatic biota than extrinsic factors such as input rates or total Hg concentrations in water and sediments. The predominance of internal factors in controlling Hg accumulation by aquatic biota is particularly true of seepage lakes where there are no inflow or outflow streams (Cope et al. 1990; Grieb et al. 1990; Wiener et al. 1990).

Recently, potential for Hg accumulation in and risk of Hg poisoning of biota inhabiting and using freshwater wetlands have received attention (e.g., Spalding et al. 1994; Sundlof et al. 1994; Gariboldi et al. 1998). While wetlands have been identified as significant sources of methyl mercury (MeHg) to boreal forest (St. Louis et al. 1994), investigations of factors controlling the bioavailability of Hg in wetlands are limited (Branfireun et al. 1996; Driscoll et al. 1998; Gilmour et al. 1998; Hurley et al. 1998; Krabbenhoft et al. 1998). Many species of wildlife, including wading birds, birds of prey, waterfowl, and mammals, feed in wetlands where physical conditions (anoxia, fluctuating water levels, and high organic carbon levels) favor methylation processes (Zillioux et al. 1993). Therefore, information on factors controlling the bioavailability of Hg in wetland systems is needed.

In this paper, we report on the first systematic survey of Hg concentrations in fish occupying southeastern depression wetlands (Carolina bays). The goal of our analyses was to assess the relative roles of trophic level, wetland morphology, hydrology, and water chemistry in controlling bioaccumulation of Hg in wetland fishes. We determined Hg concentrations in whole fishes for three species occupying three different trophic levels from nine depression wetlands on the Upper Atlantic Coastal Plain of South Carolina. We concentrate on levels in whole fish rather than muscle because fish from depression wetlands are seldom consumed by humans but are a prominent food item of many wildlife species that consume the entire fish. We specifically asked the following. (*i*) Do Hg concentrations in individual fish species vary as a function of trophic status? (*ii*) Is there significant variation in fish Hg concentrations among wetlands? (*iii*) Is variation in fish Hg concentrations among wetlands related to variation in wetland morphology, hydrology, and water chemistry? Because the physiochemical variables that we measured represent a hierarchy in which upper levels may influence lower levels, we used both a traditional regression approach and a path analysis approach to analyze relationships among fish Hg concentrations and physiochemical variables. The latter approach allowed us to assess possible complex interactions involving indirect effects.

Materials and methods

Study site and species

We sampled fish in nine depression wetlands at the Savannah River Site (SRS) on the Upper Coastal Plain of south-central South Carolina (Aiken and Barnwell counties) (Fig. 1). The numbers used to refer to wetlands are from Schalles et al. (1989) who also provided specific locations of wetlands within the SRS. The SRS is a 780-km^2 area established in the early 1950s by the U.S. Department of Energy as a nuclear production site. At the SRS, a large security buffer is maintained around relatively small industrial areas. Wetlands and their watersheds within buffer areas have not been disturbed since the site was established. Before the establishment of the SRS, land use within the watersheds of the wetlands that we studied was primarily agricultural; however, none of the wetlands were directly disturbed by agricultural practices such as plowing. Furthermore, there has been no known pollution from local anthropogenic or natural point sources.

Approximately 300 depression wetlands exist on the SRS (Kirkman et al. 1996), many of which fit the geomorphic description of Carolina bays. Fishes are found in approximately 20% of these wetlands and are restricted to wetlands located close to other aquatic habitats and that dry only during severe droughts (Snodgrass et al. 1996). Waters of depression wetlands at the SRS are acidic (pH 4.5–6.0), relatively high in organic content (DOC often >15 mg·L^{-1}), and extremely low in inorganic solutes (Newman and Schalles 1990; Pickens and Jagoe 1996).

We selected three fish species for Hg determination: lake chubsucker (*Erimyzon sucetta*), mud sunfish (*Acantharchus pomotis*), and redfin pickerel (*Esox americanus americanus*). These species are widespread among depression wetlands at the SRS (Snodgrass et al. 1996) and they represent the range of trophic levels occupied by fishes in these systems. Lake chubsucker feed by ingesting benthic detritus and associated organisms and exhibit little ontogenetic shift in diet (Sheldon and Meffe 1993). Mud sunfish feed by picking individual prey items from the substrata or water column and shift from a diet of macroinvertebrates at small sizes to a diet of macroinvertebrates and small fishes at larger sizes (Pardue 1993). Redfin pickerel are ambush predators that feed on macroinvertebrates at small sizes and shift to a diet of shrimp, crayfish, and fishes at larger sizes (Carlander 1969; Sheldon and Meffe 1993).

Sampling

We collected fishes for Hg analyses using standard minnow traps and modified hoop traps (fitted with 0.32-cm-mesh netting) from March through June of 1997. Fish were returned to the laboratory on ice. Upon return to the laboratory, fish were measured (total length in millimetres), weighed (milligrams), placed in polyethylene bags, and frozen at –4°C until they were processed and analyzed for Hg.

At the time of fish sampling, a water sample was collected into a clean 1-L polyethylene bottle. Upon return to the laboratory, pH was measured using Orion–Ross electrodes. Water was filtered using 0.4-μm-pore polycarbonate filters and split into aliquots for major cation, anion, and DOC analyses. For cation analyses, samples were preserved with trace metal grade nitric acid and major cations determined by flame atomic absorption spectroscopy. For anions, samples were stored frozen and analyzed by ion chromatography. For DOC, samples were stored frozen and analyzed using a Shimadzu TOC-500 analyzer.

Determination of maximum water depth (Z_m) and wetland area followed Snodgrass et al. (1996). We used a "drying score" as a relative measure of water level fluctuations (Snodgrass et al. 1996). Wetlands were visited five approximately evenly distributed times during 1990 and again between September 1996 and August 1997. The drying score for a wetland was calculated as the total number of wetland visits that water was present in the wetland. For the analyses presented here, our drying scores ranged from 9 (i.e., water present about 80% of the time) for wetlands with relatively variable water levels to 11 for wetlands with relatively stable water levels.

We selected six physiochemical variables to characterize the en-

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Fig. 1. Location of the SRS and study wetlands within the SRS.

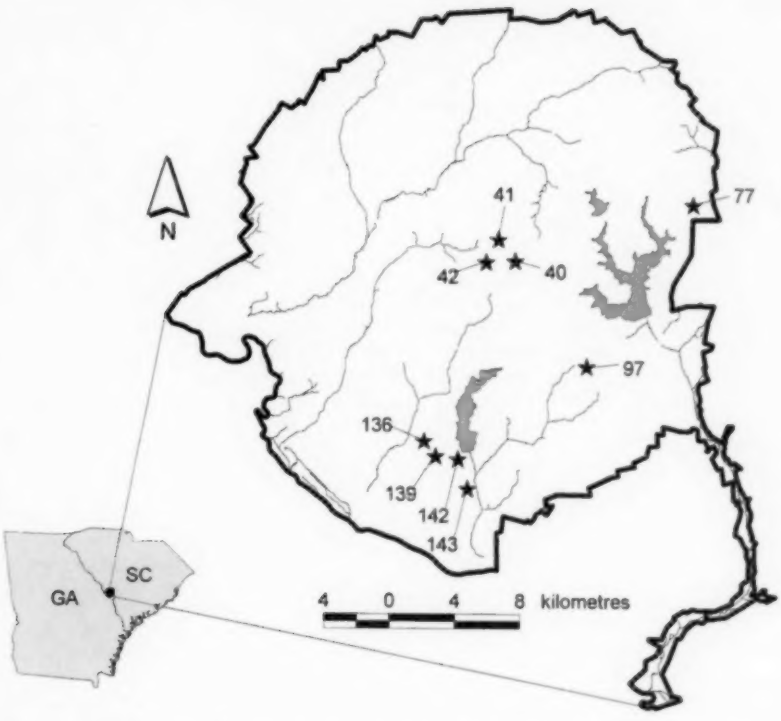

Table 1. Drying scores (DS) and morphometric and water chemistry measures for the nine southeastern depression wetlands sampled for fish.

Wetland	DS*	Area (ha)*	Z_m (cm)*	pH*	Concentration ($mg \cdot L^{-1}$) DOC*	SO_4*	K	Na	Mg	Ca	Cl	NO_3	Alkalinity ($\mu equiv. \cdot L^{-1}$)
40	11	7.52	100	5.44	16.33	0.29	0.71	0.97	0.76	1.85	2.28	0.09	120
41	10	1.00	60	5.69	17.72	0.21	0.36	1.46	1.00	2.13	1.89	0.09	163
42	11	4.89	105	5.43	12.15	0.68	0.59	1.76	0.91	1.48	3.66	0.09	86
77	9	78.20	36	4.49	15.4	0.98	0.8	1.93	0.34	0.53	4.82	0.09	0
97	9	34.02	130	4.71	10.93	0.28	0.32	1.06	0.33	0.63	2.45	0.08	0
136	9	1.22	75	4.99	15.67	0.21	0.84	1.31	0.59	0.6	3.03	0.12	8.5
139	11	1.36	67	4.45	14.61	0.78	0.33	0.64	0.46	0.65	1.17	0.08	0
142	11	5.31	94	5.56	12.08	0.19	0.3	0.93	0.72	1.99	2.58	0.43	109
143	10	12.42	33	4.60	23.19	0.28	0.23	0.61	0.46	0.43	1.62	0.09	0

Note: Variables marked with asterisks were used in analyzing variation in Hg concentrations among wetlands. Increasing drying scores correspond to increasing length of hydroperiod.

vironments of individual wetlands (Table 1). We selected this reduced set of variables because they have been associated with variation in fish Hg concentrations in lake systems (DOC, pH, sulfate, Z_m, and area) or were likely to influence levels in the wetlands that we studied (drying scores). Considering a relatively small number of variables also reduced bias associated with multiple comparisons of a single dependent variable with a number of independent variables.

Mercury analyses

We measured Hg concentrations in fish by cold-vapor atomic fluorescence (Bloom and Fitzgerald 1988; Louchouarn et al. 1993) using Brooks Rand model 2 or 3 analyzers with high-purity N_2 as a carrier gas. Before analysis, fish were freeze-dried to a constant weight, homogenized in a blender, and digested in closed Teflon vessels with 5 mL of trace metal grade or quartz-distilled nitric acid in a microwave oven. Samples were diluted to a constant volume with a 1:100 mixture of BrCl and high-purity deionized water and analyzed within 24 h. If fish mass was <0.75 g dry weight, the entire mass was digested and analyzed. For larger fish, a 0.5-g subsample of the dried, homogenized whole body was used.

Samples were digested in batches of 12, which included a blank and a standard reference material (lobster hepatopancreas or dogfish muscle, National Research Council of Canada). If the reference standard fell outside the certified range, or Hg was detected in the blank within a batch, digestion and analyses were repeated for that batch. Analyzers were calibrated daily using NIST-traceable Hg standards. Differences between replicates averaged 8% (n = 78, SD = 9%) and recovery of spikes averaged 95% (n = 40, SD = 6%). Detection and quantification limits were 0.2 and 0.7 ng, re-

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spectively. Mercury concentrations in two fish fell below quantification and detection limits. We assigned these fish values of one half the detection limit (0.1 $ng \cdot g^{-1}$) for statistical analyses.

We express all Hg concentrations in micrograms per gram of dry mass. We calculated wet mass concentrations (i.e., micrograms per gram of wet mass) by multiplying dry mass concentrations by the ratio of fish wet mass to fish dry mass. Simple linear regression ($P < 0.0001$, $R^2 = 0.94$) resulted in the equation wet mass concentration = 0.22(dry mass concentration), which can be used to convert dry mass concentrations reported here to wet mass concentrations for comparison with other studies.

Statistical analyses

We used an unbalanced, two-way ANCOVA model to compare Hg concentrations among species and wetlands. Dry mass was used as a covariate. Mercury concentrations and dry masses were $\log_{10}$ transformed before analyses to normalize their distributions. In preliminary analyses, we included interaction terms in the model but dropped nonsignificant ($P > 0.05$) interaction terms from the final model. We used Tukey's multiple range test to compare means among species. When weight was a significant ($P \leq 0.05$ based on ANCOVA models) predictor of Hg concentrations, we calculated least-squared means (LSMs) for each wetland. LSMs are the estimated mean values of class variables when all other variables in the model are held at their mean levels (Searle et al. 1980). Comparing LSMs among wetlands allowed us to remove variation associated with size and investigate relationships among wetland morphology, hydroperiod, and water chemistry and the remaining variation in Hg concentrations. All ANCOVAs were carried out using the GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.). Inspection of residual plots from ANCOVA models for individual species indicated that the assumptions of normality, linearity, and homoscedatisity were met.

We used two methods to investigate relationships among fish Hg concentrations and physiochemical variables, linear regression and path analysis. Linear regression allowed us to reduce the set of correlated independent environmental variables to a few that exhibited significant correlations with Hg concentrations while retaining the units of measure of the variables in the model. Path analysis (Wright 1934) is similar to regression analysis, but the path diagram allows comparison of the relative importance and direction of relationships among correlated independent variables, evaluation of indirect effects, and a more explicit statement of the relationships between independent variables. However, because regression coefficients are standardized in path analysis, units are lost from the analysis (Johnson and Wichern 1988). Fjeld and Rognerud (1993) provided an example of the use of path analysis in modeling Hg concentrations in fish and discussed the advantages of this approach. We used means (when concentrations were not related to fish mass) or LSMs (when concentrations were related to fish mass) of $\log_{10}$-transformed Hg concentrations as the dependent variable in all analyses of relationships among physiochemical variables and fish Hg concentrations. We inspected probability plots (Sokal and Rohlf 1991) to identify environmental variable distributions that did not meet the normality assumptions. Wetland area, DOC, and sulfate exhibited skewed distributions and were $\log_{10}$ transformed before analyses.

We carried out regression analyses in two steps. First, as an exploratory step, we fit all possible one and two independent variable models and calculated an adjusted R^2 ($\bar{R}^2$) for each model. We restricted our analyses to one or two independent variables to avoid overfitted models that may result when the number of observations is low in relation to the number of independent variables in the model (Kleinbaum et al. 1988). Second, we chose the most frequently occurring independent variables in the one- and two-variable models to test for a significant relationship between Hg concentrations and environmental variables using a multivariate approach to regression (MANOVA). MANOVA was carried out using the PROC REG procedure of SAS (SAS Institute, Inc., Cary, N.C.). MANOVA was used to test for significant relationships between selected environmental variables and Hg concentrations to avoid bias associated with multiple tests of correlated dependent variables. We examined plots of residuals and partial regression leverage plots to assure linear relationships among dependent and independent variables and that one observation did not exhibit overdue influence on the results.

We used path analysis to evaluate models in which morphological and hydrological factors were exogenous variables (determined by factors outside the system of linear equations) and water chemistry and Hg concentrations were endogenous variables (determined by factors inside the system of linear equations). We selected a subset of variables based on the results of regression analyses and compared two models for each species: (*i*) a full model where all morphological and hydrological variables in the model could have direct effects and (*ii*) a reduced model with fewer direct effects of morphological and hydrological variables. We used the maximum-likelihood method of the CALIS procedure of SAS (SAS Institute, Inc., Cary, N.C.) to estimate standardized regression coefficients for models. To investigate the possibility that the effects of morphological and hydroperiod variables were expressed better or equally well as indirect effects, we used a χ^2 statistic to test the null hypothesis that adding direct effects of morphological and hydroperiod variables would not increase the predictive ability of models. To increase the likelihood that our estimates were the global optimum (and not a local optimum), we conducted estimations 10 times with 10 different randomly chosen initial parameter estimates. In all cases, each estimate was the same, suggesting that our solutions were at the global optimum.

Results

We collected and measured Hg concentrations in 248 fishes (Table 2). Mercury concentrations ranged from <0.01 to 1.75 $\mu g \cdot g^{-1}$ in lake chubsucker, from 0.13 to 1.53 $\mu g \cdot g^{-1}$ in mud sunfish, and from <0.01 to 1.90 $\mu g \cdot g^{-1}$ in redfin pickerel. Fish Hg concentrations varied significantly among species and wetlands (Table 3), and variation was greater among wetlands than among species. Geometric LSM Hg concentrations (i.e., back-transformed LSMs from the ANCOVA) ranged from 0.28 to 0.45 $\mu g \cdot g^{-1}$ among species. This range was smaller than any of the ranges of geometric means of species among wetlands (Table 2). The slopes of the lines relating Hg concentrations to fish mass differed among species, as indicated by the significant interaction between species and mass (Table 3) in the ANCOVA model. Among lake chubsucker, the slope of the line relating Hg concentrations to fish mass was not significantly different from zero ($P = 0.108$). Among mud sunfish ($P \leq 0.013$) and redfin pickerel ($P = 0.001$), the slopes of the lines relating Hg concentrations to fish mass were significant, and the slope was greatest among redfin pickerel (Fig. 2). However, regressions of fish Hg concentrations on mass accounted for little of the variation among mud sunfish ($r^2 = 0.07$) and redfin pickerel ($r^2 = 0.12$). Use of length in regression analyses produced similar results. Geometric mean Hg concentrations increased from lake chubsucker (0.28 $\mu g \cdot g^{-1}$) to mud sunfish (0.37 $\mu g \cdot g^{-1}$) to redfin pickerel (0.45 $\mu g \cdot g^{-1}$), as expected based on their diets. Based on these results, we investigated differences in Hg concentrations among wetlands using means for lake chubsucker and LSMs for mud sunfish and redfin pickerel.

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Table 2. Sample size (n), mean weight and length, and geometric mean Hg concentrations in whole fish of three species (ES, *Erimyzon sucetta*; AP, *Acantharchus pomotis*; EA, *Esox americanus americanus*) collected from nine southeastern depression wetlands.

	n			Mean (SD) dry weight (g)			Mean (SD) standard length (mm)			Geometric mean Hg (μg·g dry weight^{-1})		
Wetland	ES	AP	EA	ES	AP	EA	ES	AP	EA	ES	AP	EA
40	10	11	13	2.12 (2.77)	3.84 (1.77)	3.03 (1.81)	76.80 (40.77)	94.27 (14.87)	121.31 (29.41)	0.18	0.19	0.26
41	10	11	9	8.89 (2.13)	4.63 (4.17)	4.21 (2.12)	147.30 (13.4)	85.82 (35.22)	136.00 (24.61)	0.32	0.46	0.49
42	10		7	7.49 (5.24)		2.25 (1.13)	135.00 (28.64)		105.14 (22.38)	0.19		0.32
77	12	11	9	5.62 (3.75)	0.66 (0.31)	0.89 (1.28)	122.92 (23.52)	49.64 (7.61)	76.56 (28.43)	0.63	0.61	1.05
97	8	10	6	9.26 (12.53)	1.08 (0.21)	2.37 (1.75)	116.88 (65.61)	54.50 (3.34)	99.00 (26.88)	0.27	0.27	0.24
136	9	10	11	2.14 (1.55)	6.99 (6.96)	4.16 (4.58)	85.56 (27.9)	92.20 (30.67)	125.36 (37.98)	0.33	0.49	0.68
139	10	9	11	4.45 (2.46)	5.06 (0.96)	0.49 (0.51)	109.30 (19.89)	100.22 (5.02)	67.45 (24.77)	0.28	0.47	0.35
142	5	15	11	0.08 (0.02)	1.23 (1.37)	1.94 (2.82)	34.80 (3.27)	56.87 (17.48)	97.36 (36.15)	0.20	0.34	0.31
143		10	10		8.00 (3.80)	3.24 (2.86)		108.60 (15.03)	118.20 (45.89)		0.51	0.46
Overall	74	87	87	3.74 (4.02)	5.28 (5.64)	2.53 (2.67)	78.56 (22.81)	108.62 (43.34)	105.68 (37.8)	0.28	0.37	0.45

Note: Standard deviations are given in parentheses for weight and length. Geometric mean Hg concentrations were standardized to mean dry mass for *A. pomotis* and *E. americanus americanus*. Geometric means were not standardized for *E. sucetta* because mass was not related to Hg concentrations in this species.

Table 3. Results of two-way ANCOVA of the effects of wetland and species on Hg concentrations (μg·g dry mass^{-1}) in fishes from southeastern depression wetlands and one-way ANCOVA of the effects of wetland on Hg concentrations in individual species.

Source	df	SS	F	P
All species				
$\log_{10}$(dry mass)	1	1.18	21.45	<0.001
Wetland	8	6.42	14.65	<0.001
Species	2	1.08	9.82	<0.001
$\log_{10}$(dry mass) × species	2	0.74	6.71	0.002
Error	234	12.82		
Lake chubsucker				
$\log_{10}$(dry mass)	1	0.01	0.07	0.797
Wetland	7	2.30	4.18	<0.001
Error	65	5.11		
Mud sunfish				
$\log_{10}$(dry mass)	1	0.09	4.10	0.046
Wetland	7	2.30	14.51	<0.001
Error	78	1.77		
Redfin pickerel				
$\log_{10}$(dry mass)	1	0.92	13.73	<0.001
Wetland	8	3.48	6.46	<0.001
Error	77	5.18		

Note: The covariate used in all analyses was fish dry mass. Nonsignificant ($P > 0.05$) interaction terms were dropped from the final models.

Geometric mean fish Hg concentrations for individual wetlands are given in Table 2. In no case was the interaction between wetlands and mass significant in the ANCOVA models for individual species (Table 3), indicating relatively homogeneous slopes among wetlands. Among wetlands, geometric mean Hg concentrations for individual species were highly correlated ($r = 0.82$ for lake chubsucker and mud sunfish, $r = 0.88$ for lake chubsucker and redfin pickerel, and $r = 0.83$ for mud sunfish and redfin pickerel). Geometric mean Hg concentration for individual species was lowest in wetland 40 and highest in wetland 77.

Variation in fish Hg concentrations among wetlands was correlated with wetland morphology, hydrology, and water chemistry, with fish Hg concentrations most strongly related to Z_m and drying scores (Fig. 3). Drying scores, morphometric data, and select water chemistry variables for the wetlands are given in Table 1. One- and two-variable regression models with the highest $\bar{R}^2$ are given in Table 4. For all three species, Z_m produced the one-variable model with the highest $\bar{R}^2$ (range 0.50–0.62). All other one-variable models had $\bar{R}^2$ values <0.25 except the model for lake chubsucker that contained drying scores ($\bar{R}^2 = 0.48$). Among two-variable regression models, models containing Z_m and drying scores had the highest $\bar{R}^2$ values for lake chubsucker ($\bar{R}^2 = 0.91$) and redfin pickerel ($\bar{R}^2 = 0.73$). For mud sunfish, the two-variable model with the highest $\bar{R}^2$ contained Z_m and DOC. Other two-variable models with $\bar{R}^2$ values within 0.10 of the highest $\bar{R}^2$ values also contained a combination of Z_m, drying scores, and DOC. MANOVA indicated significant relationships between fish Hg concentrations and Z_m ($P = 0.001$), drying scores ($P = 0.002$), and DOC ($P = 0.017$).

Based on the results of the regression analyses, we included Z_m, drying score, and DOC as independent environmental variables in path analysis models. Our full model contained direct effects of all three variables on Hg concentrations and an indirect effect of Z_m on DOC. The set of structural equations for this model were

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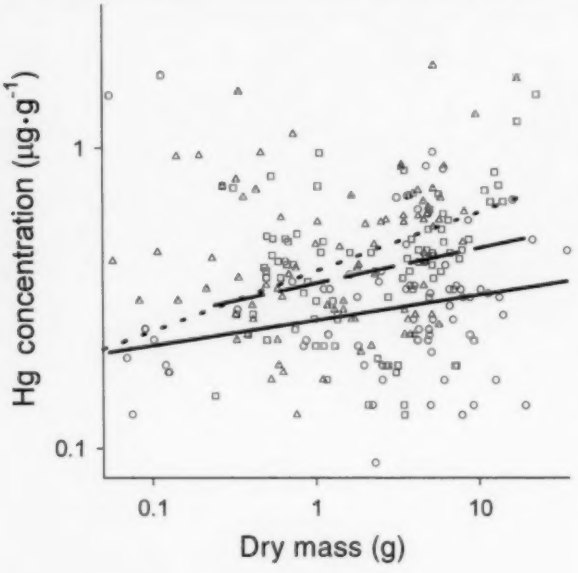
Fig. 2. Relationship between fish dry mass and Hg concentrations for lake chubsucker (circles and solid line), mud sunfish (squares and long-dashed line), and redfin pickerel (triangles and short-dashed line) from nine southeastern depression wetlands. Lines are simple linear regressions.



$$(1) \quad \log_{10}(\mathrm{DOC}) = \beta_1(Z_m) + \zeta_1$$

$$(2) \quad \text{Fish Hg concentration} = \beta_2(Z_m) + \beta_3(\mathrm{DS}) + \beta_4(\log_{10}(\mathrm{DOC})) + \zeta_2$$

where DS is drying score. We included an effect of Z_m, but not drying score, on DOC because Z_m and DOC were correlated ($r = -0.796$) but drying score and DOC were not ($r = -0.071$). Our reduced model did not contain direct effects of Z_m.

The dominant role of morphology and hydroperiod in controlling variation in fish Hg concentrations among wetlands was further supported by path analyses. The fit of the full path models was good for all three species. Goodness-of-fit indices were 0.99, 0.98, and 0.99 and coefficients of determination (r^2) were 0.97, 0.88, and 0.87 for lake chubsucker, mud sunfish, and redfin pickerel, respectively. For all full models, χ^2 tests indicated that adding additional effects to the models would not increase their predictive ability ($P > 0.578$). In contrast, χ^2 tests indicated that adding additional effects to the reduced models would increase their predictive ability ($P < 0.005$).

In the full models for all species, Z_m had the greatest effect (i.e., largest standardized path coefficients) (Fig. 4). In all three cases, the effects of Z_m were negative, with path coefficients less than or equal to –0.81. The effects of drying score and DOC also were negative, but their relative strength varied among the full models. In the model for lake chubsucker, the effects of drying score were greater than those of DOC, while in the mud sunfish model, the effects of DOC were greater than those of drying score. In the redfin pickerel model, the effects of drying score and DOC were similar (Fig. 4). The indirect effects of Z_m (through its influence of DOC) can be calculated by multiplying standardized path coefficients and were positive in all cases, ranging from 0.18 in the lake chubsucker model to 0.41 in the mud sunfish model. Therefore, the indirect effects of Z_m contrasted with the direct effects of Z_m.

Discussion

Mercury does not appear to pose an acute risk to fish and wildlife in the wetlands that we studied but may pose a chronic risk. Mercury levels in the three species of fish that we investigated were well below levels reported to be associated with MeHg toxicity in fish (Wiener and Spry 1996). However, when we calculated wet mass concentrations, 34% of the fish had Hg concentrations that exceeded the 0.1 μg·g wet mass^{-1} considered by the U.S. Fish and Wildlife Service (Eisler 1987) to be a safe standard for consumption by sensitive avian species. Given that accumulation of Hg in wetland fishes may pose a risk to wildlife that feed on fish, it is important to understand the factors controlling variation in Hg accumulation among species and wetlands.

Influence of trophic level

Feeding habits have long been recognized as influencing Hg levels in aquatic organisms (Wiener and Spry 1996). Methyl mercury is the primary form of Hg in fish tissues (Grieb et al. 1990; Bloom 1992) because MeHg uptake by fish is relatively efficient and elimination is inefficient. Because MeHg biomagnifies in aquatic food webs (Kidd et al. 1995), fishes that exhibit ontogenetic shifts in diet from small, lower trophic level prey to larger, higher trophic level prey will be exposed to higher concentrations of Hg in their diet as they age. If age is associated with size (as it is with most fishes), accumulation rates of Hg should increase with size in species with ontogenetic shifts in diet. We studied three fish species that exhibit different degrees of ontogenetic shifts in diet to test the application of this idea to depression wetlands.

Our results suggest that fish species that shift from a diet of small, lower trophic level prey to larger, higher trophic level prey bioaccumulate Hg to a larger degree than species that do not exhibit such a dietary shift. Concentrations of Hg in lake chubsucker, a species that feeds by ingesting detritus and algae (and associated organisms) throughout its life, were not significantly related to weight across the size range of fish that we investigated, suggesting low rates of bioaccumulation of Hg with age for this species. In contrast, concentrations of Hg in both mud sunfish and redfin pickerel were positively related to size. Both of these species shift from a diet of small invertebrates as young fish to a diet of larger invertebrates and vertebrates as adults. Because diet is the primary route of Hg uptake by fish (Wiener and Spry 1996; Hall et al. 1997) and the species that we studied have similar longevity and growth rates (Carlander 1969; Pardue 1993), our results suggest that Hg biomagnification through higher trophic levels occurs and deserves further investigation in southeastern depression wetlands.

Influence of wetland morphology and hydroperiod

We observed negative correlations between fish Hg concentrations and Z_m and drying scores, indicating highest Hg levels in fish from shallow wetlands with relatively large wa-

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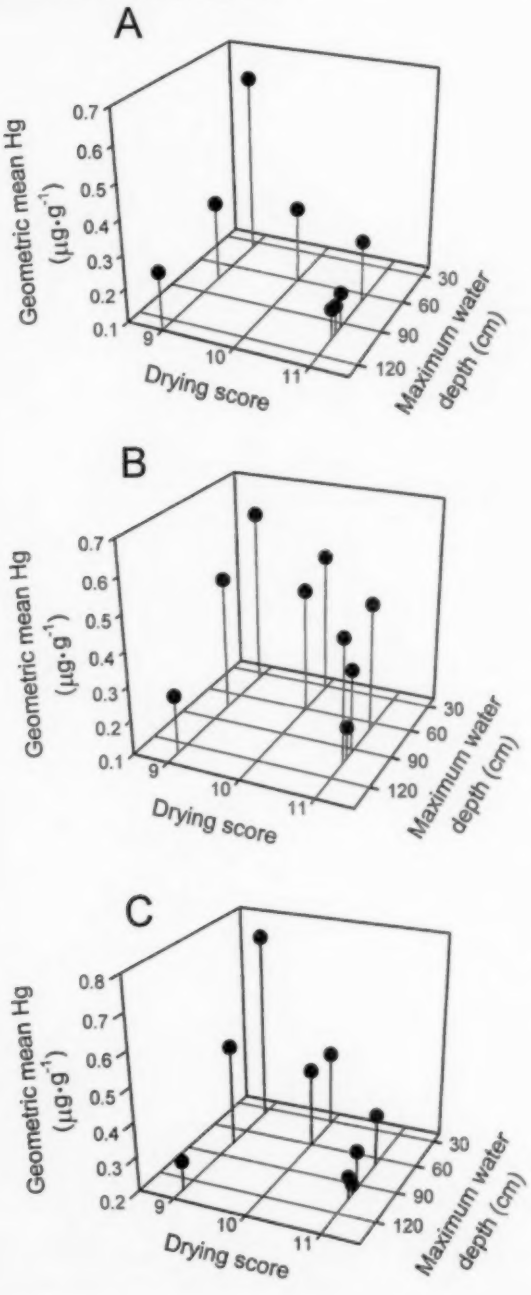
Fig. 3. Relationships among maximum depth, drying score, and (A) lake chubsucker, (B) mud sunfish, and (C) redfin pickerel geometric mean Hg concentrations.



ter level fluctuations. Caldwell and Canavan (1998) reported that concentration of total Hg and MeHg in water and sediment were highest at a site in a New Mexico reservoir that experienced periodic drying and flooding compared with five sites in deeper water. While most studies of flooding effects on Hg dynamics have focused on a single flooding event such as the creation of a reservoir or flooding of a wetland (Bodaly et al. 1997), our results, as well as those of Caldwell and Canavan (1998), suggest that intermittent or periodic flooding associated with changes in water levels in reservoirs and wetlands can also enhance MeHg production and (or) bioavailability.

The relationship between elevated Hg concentrations in fish and periodic flooding is undoubtedly related to many complex and interacting factors involving production and breakdown of multiple Hg species. We hypothesize that a key process in periodically flooded systems involves release of bound Hg from sediments during dry periods and uptake by biota when sediments are reflooded. Mercury may become bound to sulfides and organic and inorganic particles in sediments during anoxic periods. Such anoxic conditions also favor the methylation of Hg, particularly in the surficial layers of sediment (Gilmour et al. 1998). When wetlands dry, sediments become aerobic, physical properties change, and bounded Hg and MeHg maybe released (Lodenius 1990). Upon reflooding, Hg and MeHg are leached from sediments and are available to the aquatic food web until an equilibrium under anoxic sediment conditions is reestablished. Thus, periodic drying and flooding results in alternating periods of Hg methylation and release. If this is correct, one might speculate that, other factors being equal, methylation and release of Hg would be enhanced in a shallow, saucer-shaped wetland relative to a deeper one with a steeper shoreline. Our drying score is a measure of complete drying and water level fluctuation and was negatively correlated with fish Hg concentrations, as would be expected under this scenario. Moreover, the Z_m of a wetland controls the amount of substrate that would be exposed with a given decrease in water level, so a negative relationship between Z_m and fish Hg concentration such as we observed would also be predicted.

Several studies have related lake morphometric features to fish Hg concentrations in northern lakes, but these relationships are attributed to the effects of morphometric features on input and retention of Hg or effects on Hg cycling in the water column. A positive relationship between lake Z_m and Hg concentrations in sediments of Norwegian lakes and a negative relationship between drainage basin area to lake volume ratios and fish Hg concentrations among Canadian lakes were attributed to the effects of these factors on Hg input to and retention of Hg by lakes (Sun and Hitchin 1990; Fjeld and Rognerud 1993). Bodaly et al. (1993) suggested that a negative correlation between Hg concentrations in upper trophic level fishes and size of Canadian Shield lakes resulted from higher average epilimnetic temperatures in smaller lakes and their effects on methylation and fish metabolic rates.

Influence of water chemistry

We had originally expected morphological and hydrologic variables to affect water chemistry variables which would directly influence fish Hg concentrations. This view promoted our path analyses. However, we found few relationships between water chemistry variables and fish Hg concentrations. In contrast, studies of lake systems have often found relationships between the water chemistry variables that we measured and fish Hg concentrations. In northern drainage

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Table 4. One- and two-variable regression models with the highest $\bar{R}^2$ values for all possible models containing a combination of one or two independent environmental variables.

Species and model	Variable	Coefficient	SE	*P*	$\bar{R}^2$
Lake chubsucker					
One variable	Constant	–0.181	0.139	0.242	0.50
	Maximum depth (cm)	–0.005	0.002	0.029	
Two variables	Constant	0.868	0.202	0.008	0.91
	Maximum depth (cm)	–0.004	0.001	0.003	
	Drying score	–0.110	0.020	0.003	
Mud sunfish					
One variable	Constant	–0.093	0.095	0.362	0.62
	Maximum depth (cm)	–0.004	0.001	0.012	
Two variables	Constant	1.285	0.671	0.114	0.76
	Maximum depth (cm)	–0.007	0.001	0.007	
	$\log_{10}$(DOC) ($mg \cdot L^{-1}$)	–0.440	0.213	0.093	
Redfin pickerel					
One variable	Constant	0.008	0.127	0.950	0.56
	Maximum depth (cm)	–0.005	0.002	0.013	
Two variables	Constant	0.971	0.422	0.061	0.73
	Maximum depth (cm)	–0.005	0.001	0.010	
	Drying score	–0.100	0.042	0.057	

Note: Dependent variables were mean $\log_{10}$-transformed Hg concentrations and were standardized to mass for mud sunfish and redfin pickerel. Transformed means were not standardized for lake chubsucker because mass was not related to Hg concentrations in this species.

and seepage lakes, fish Hg concentrations are often negatively correlated with pH (Cope et al. 1990; Grieb et al. 1990; Sun and Hitchin 1990; Wiener et al. 1990). We found that pH was not correlated with fish Hg concentrations in the depression wetlands that we studied. This result may be due to the narrow range and generally acidic pH of the wetlands that we studied (about 4.5–5.5). All of the studies reporting correlations between pH and Hg concentrations in fish include much larger pH ranges (about 5.5–7.5). Because pH rarely exceeds 6.0 in depression wetlands of the Atlantic Coastal Plain (Newman and Schalles 1990), pH is probably not a significant factor influencing fish Hg concentrations in depression wetlands.

It has also been suggested that correlations among lake pH and fish Hg concentrations may result from addition of sulfate as a result of acid rain (Winfrey and Rudd 1990). Sulfate enters aquatic systems (with no point sources of pollutant) in acid rain in which the primary strong acid is sulfuric acid. Sulfate has been shown to increase MeHg concentrations in sediments and water by stimulating sulfate-metabolizing bacteria and their methylation activities under anaerobic conditions (Gilmour et al. 1992) and indirectly by decreasing pH. However, the effects of sulfate may be limited by toxic sulfide byproducts of sulfate metabolism and reduced bioavailability of Hg from Hg binding to free sulfide under anaerobic conditions (Winfrey and Rudd 1990; Dyrssen and Wedborg 1991). In the wetlands that we studied, dissolved oxygen levels are near zero in waters overlaying wetland sediments for large portions of the day (J.W. Snodgrass, personal observation). Therefore, conditions exist that favor methylation and sulfide production by sulfate-metabolizing bacteria. However, we found no relationship between sulfate levels and fish Hg concentrations, possibly because sulfate levels in the wetlands that we studied (<1.0 $mg \cdot L^{-1}$) were below levels that affect methylation rates (greater than about 10 $mg \cdot L^{-1}$) in laboratory and field experiments (Winfrey and Rudd 1990; Gilmour et al. 1992). Sulfate levels in other depression wetlands of the Atlantic Coastal Plain are within the range (17–22 $mg \cdot L^{-1}$; Newman and Schalles 1990) known to influence methylation rates. Studies including wetlands having higher sulfate levels are needed to fully define the relationship between sulfate levels and Hg concentrations in fish inhabiting depression wetlands of the Atlantic Coastal Plain.

Positive and negative correlations of fish Hg concentrations and DOC levels have been reported among drainage and seepage lakes, respectively (Grieb et al. 1990; Wren et al. 1991). We found a negative relationship between mud sunfish Hg concentration and DOC levels. Negative correlations between fish Hg concentrations and DOC levels can result from DOC binding of ionic Hg (Hg^{2+}) and MeHg, reducing their availability for methylation and uptake by fishes, respectively (Winfrey and Rudd 1990). Furthermore, increased DOC levels have been shown to reduce MeHg uptake via the gills under basic ($pH \approx 8.0$) water conditions (Ramamoorthy and Blumhagen 1984; Choi et al. 1998).

It is unclear why DOC levels were more strongly related to Hg concentrations in mud sunfish, but this may be related to differences in diet among the species studied. Mud sunfish obtain a substantial portion of their diet from the water column, especially when they are small and feed on zooplankton (Pardue 1993). Lake chubsucker and redfin pickerel feed mainly on benthic organisms. Binding of Hg by DOC is most likely to influence bioavailability in the water column portion of the food web.

Finally, we did not investigate the role of differences in Hg input to individual wetlands, a factor that appears to be important in northern drainage lakes (e.g., Evans 1986; Cope et al. 1990). However, because our wetlands all have small watersheds with no streams draining into them and are in

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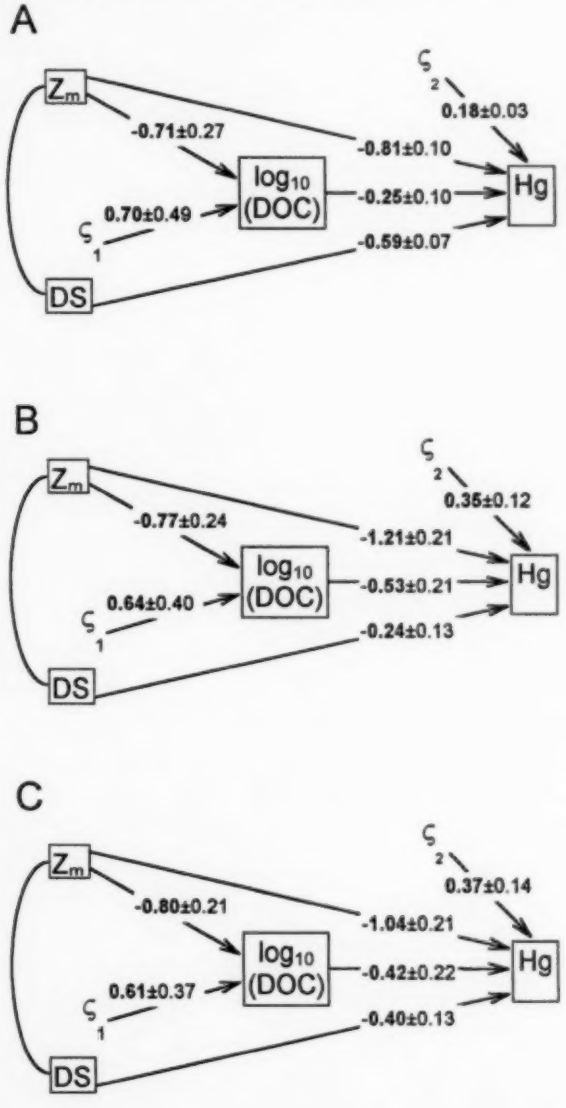
Fig. 4. Path diagrams describing Hg concentrations in (A) lake chubsucker, (B) mud sunfish, and (C) redfin pickerel as a function of wetland morphology, hydrology, and water chemistry. Arrows indicate suggested causal relationships. Path coefficients and their standard errors are given on the arrows and indicate the direction and magnitude of effects. Residual effects are indicated by ζ. DS, drying score; Hg, fish Hg concentration.



close proximity, it seems likely that variation among wetlands in atmospheric and water-borne input is probably minimal.

Conclusions

We found significant variation in Hg concentrations among species and wetlands in fish occupying southeastern depression wetlands. Variation among species was related to trophic level but was less than variation among wetlands. Variation in fish Hg concentrations among wetlands was related to variation in morphometric and hydrological characteristics, specifically Z_m and hydroperiod. Our findings lead us to hypothesize that the effects of repeated flooding on Hg cycling in sediments are an important process controlling the bioavailability of Hg in wetlands. Additionally, the positive correlation that we observed between degree of water level fluctuation and fish Hg concentrations suggests that management of water level fluctuations can be used to control the bioavailability of Hg in aquatic systems.

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References

Bloom, N.S. 1992. On the chemical forms of mercury in edible fish and marine invertebrates. Can. J. Fish. Aquat. Sci. **49**: 1010–1017.

Bloom, N.S., and Fitzgerald, W.F. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapor atomic fluorescence detection. Anal. Chim. Acta, **208**: 151–161.

Bodaly, R.A., Rudd, J.W.M., Fudge, R.J.P., and Kelly, C.A. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. Can. J. Fish. Aquat. Sci. **50**: 980–987.

Bodaly, R.A., St. Louis, V.L., Paterson, M.J., Fudge, R.J.P., Hall, B.D., Rosenberg, D.M., and Rudd, J.W.M. 1997. Bioaccumulation of mercury in the aquatic food chain in newly flooded areas. *In* Metal ions in biological systems. Vol. 34. Mercury and its effects on environment and biology. *Edited by* A. Sigel and H. Sigel. Marcel Dekker, Inc., New York. pp. 259–287.

Branfireun, B.A., Heyes, A., and Roulet, N.T. 1996. The hydrology and methylmercury dynamics of a Precambrian Shield headwater peatland. Water Resour. Res. **32**: 1785–1794.

Caldwell, C.A., and Canavan, C.M. 1998. Spatial and temporal distribution of mercury in Caballo and Elephant Butte reservoirs, Sierra County, New Mexico. WRRI Tech. Completion Rep. 306, New Mexico Water Resources Research Institute, Las Cruces, N.M.

Carlander, K.D. 1969. Handbook of freshwater fisheries biology. Vol. 1. Iowa State University Press, Ames, Iowa.

Choi, M.H., Cech, J.J., Jr., and Lagunas-Solar, M.C. 1998. Bioavailability of methylmercury to Sacramento blackfish (*Orthodon microlepidotus*): dissolved organic carbon effects. Environ. Toxicol. Chem. **17**: 695–701.

Clarkson, T.W. 1990. Human health risk from methylmercury in fish. Environ. Toxicol. Chem. **9**: 957–961.

Cope, W.G., Wiener, J.G., and Rada, R.G. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: relation to lake characteristics. Environ. Toxicol. Chem. **9**: 931–940.

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Driscoll, C.T., Holsapple, J., Schofield, C.L., and Munson, R. 1998. The chemistry and transport of mercury in a small wetland in the Adirondack region of New York, USA. Biogeochemistry, **40**: 137–146.

Dyrssen, D., and Wedborg, M. 1991. The sulphur–mercury(II) system in natural waters. Water Air Soil Pollut. **56**: 507–519.

Eisler, R. 1987. Mercury hazards to fish, wildlife and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. 85.

Evans, R.D. 1986. Sources of mercury contamination in the sediments of small headwater lakes in south-central Ontario, Canada. Arch. Environ. Contam. Toxicol. **15**: 505–512.

Fjeld, E., and Rognerud, S. 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. Can. J. Fish. Aquat. Sci. **50**: 1158–1167.

Gariboldi, J.C., Jagoe, C.H., and Bryan, A.L., Jr. 1998. Dietary exposure to mercury in nestling wood storks (*Mycteria americana*) in Georgia. Arch. Environ. Contam. Toxicol. **34**: 398–405.

Gilmour, C.C., Henry, E.A., and Mitchell, R. 1992. Sulfate stimulation of mercury methylation in freshwater sediment. Environ. Sci. Technol. **26**: 2281–2287.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A., and Stordal, M.C. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry, **40**: 327–345.

Grieb, T.M., Driscoll, C.T., Gloss, S.P., Schofield, C.L., Bowie, G.L., and Porcella, D.B. 1990. Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Environ. Toxicol. Chem. **9**: 919–930.

Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.M., and Rosenberg, D.M. 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut. **100**: 13–24.

Hurley, J.P., Krabbenhoft, D.P., Cleckner, L.B., Olson, M.L., Aiken, G.R., and Rawlik, P.S., Jr. 1998. System controls on the aqueous distribution of mercury in the northern Florida Everglades. Biogeochemistry, **40**: 293–310.

Johnson, R.A., and Wichern, D.W. 1988. Applied multivariate statistical analysis. Prentice Hall, Englewood Cliffs, N.J.

Kidd, K.A., Hesslein, R.H., Fudge, R.J.P., and Hallard, K.A. 1995. The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in freshwater organisms. Water Air Soil Pollut. **80**: 1011–1015.

Kirkman, L.K., Lide, R.F., Wein, G., and Sharitz, R.R. 1996. Vegetation change and land-use legacies of depression wetlands of the western Coastal Plain of South Carolina: 1951–1992. Wetlands, **16**: 564–576.

Kleinbaum, D.G., Kupper, L.I., and Muller, K.E. 1988. Applied regression analysis and other multivariate methods. 2nd ed. PWS-Kent Publishing Co., Boston, Mass.

Krabbenhoft, D.P., Hurley, J.P., Olson, M.L., and Cleckner, L.B. 1998. Diel variability of mercury phase and species distributions in the Florida Everglades. Biogeochemistry, **40**: 311–325.

Lodenius, M. 1990. Sorption of mercury in soils. *In* Encyclopedia of environmental control and technology. Vol. 4. Hazardous waste containment and treatment. *Edited by* P.N. Cheremisinoff. Gulf Publishing Co., Houston, Tex. pp. 339–353.

Louchouarn, P., Lucotte, M., Mucci, A., and Pichet, P. 1993. Geochemistry of mercury in two hydroelectric reservoirs in Quebec, Canada. Can. J. Fish. Aquat. Sci. **50**: 269–281.

Newman, M.C., and Schalles, J.F. 1990. The water chemistry of Carolina bays: a regional survey. Arch. Hydrobiol. **118**: 147–168.

Pardue, G.B. 1993. Life history and ecology of the mud sunfish (*Acantharchus pomotis*). Copeia, **1993**: 533–540.

Pickens, R.M., and Jagoe, C.H. 1996. Relationships between precipitation and surface water chemistry in three Carolina bays. Arch. Hydrobiol. **137**: 187–209.

Ramamoorthy, S., and Blumhagen, K. 1984. Uptake of Zn, Cd, and Hg by fish in the presence of competing compartments. Can. J. Fish. Aquat. Sci. **41**: 750–756.

Schalles, J.F., Sharitz, R.R., Gibbons, J.W., and Knox, J.N. 1989. Carolina bays of the Savannah River Plant. National Environmental Research Park Program No. SRO-NERP-18. Savannah River Ecology Laboratory, Aiken, S.C.

Searle, S.R., Speed, F.M., and Milliken, G.A. 1980. Populations marginal means in the linear model: an alternative to least squares means. Am. Stat. **34**: 216–221.

Sheldon, A.L., and Meffe, G.K. 1993. Multivariate analysis of feeding relationships of fishes in blackwater streams. Environ. Biol. Fishes, **37**: 161–171.

Snodgrass, J.W., Bryan, A.L., Jr., Lide, R.F., and Smith, G.M. 1996. Factors affecting the occurrence and structure of fish assemblages in isolated wetlands of the upper coastal plain, U.S.A. Can. J. Fish. Aquat. Sci. **53**: 443–454.

Sokal, R.R., and Rohlf, F.J. 1991. Biometry. W.H. Freeman and Company, New York.

Spalding, M.G., Bjork, R.D., Powell, G.V.N., and Sundlof, S.F. 1994. Mercury and causes of death in Great White Herons. J. Wildl. Manage. **58**: 735–739.

St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Beaty, K.G., Bloom, N.S., and Flett, R.J. 1994. Importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Can. J. Fish. Aquat. Sci. **51**: 1065–1076.

Sun, K., and Hitchin, G. 1990. Interrelationships between mercury levels in yearling yellow perch, fish condition and water quality. Water Air Soil Pollut. **650**: 255–265.

Sundlof, S.F., Spalding, M.G., Wentworth, J.D., and Steible, C.K. 1994. Mercury in livers of wading birds (Ciconiformes) in southern Florida. Arch. Environ. Contam. Toxicol. **27**: 299–305.

Thompson, D.R. 1996. Mercury in birds and terrestrial mammals. *In* Environmental contaminants in wildlife: interpreting tissue concentrations. *Edited by* W.N. Beyer, G.H. Hienz, and A.W. Redmon-Norwood. CRC Press, Boca Raton, Fla. pp. 341–356.

Wiener, J.G., and Spry, D.J. 1996. Toxicological significance of mercury in freshwater fish. *In* Environmental contaminants in wildlife: interpreting tissue concentrations. *Edited by* W.N. Beyer, G.H. Hienz, and A.W. Redmon-Norwood. CRC Press, Boca Raton, Fla. pp. 297–340.

Wiener, J.G., Martini, R.E., Sheffy, T.B., and Glass, G.E. 1990. Factors influencing mercury concentrations in walleyes in northern Wisconsin lakes. Trans. Am. Fish. Soc. **119**: 862–870.

Winfrey, M.R., and Rudd, J.W.M. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes: a review. Environ. Toxicol. Chem. **9**: 853–869.

Wren, C.D., Scheider, W.A., Wales, D.L., Muncaster, B.W., and Gray, I.M. 1991. Relation between mercury concentrations in walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*) in Ontario lakes and influence of environmental factors. Can. J. Fish. Aquat. Sci. **48**: 132–139.

Wright, S. 1934. The method of path coefficients. Ann. Math. Stat. **5**: 161–215.

Zillioux, E.J., Porcella, D.B., and Benoit, J.M. 1993. Mercury cycling and effects in freshwater wetland ecosystems. Environ. Toxicol. Chem. **12**: 2245–2264.

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Comparison of parameter estimation methods for detecting climate-induced changes in productivity of Pacific salmon (*Oncorhynchus* spp.)

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Abstract: Pacific salmon (*Oncorhynchus* spp.) populations can experience persistent changes in productivity, possibly due to climatic shifts. Management agencies need to rapidly and reliably detect such changes to avoid costly suboptimal harvests or depletion of stocks. However, given the inherent variability of salmon populations, it is difficult to detect changes quickly, let alone forecast them. We therefore compared three methods of annually updating estimates of stock–recruitment parameters: standard linear regression, Walters' bias-corrected regression, and a Kalman filter. We used Monte Carlo simulations that hypothesized a wide range of future climate-induced changes in the Ricker *a* parameter of a salmon stock. We then used each parameter estimation method on the simulated stock and recruitment data and set escapement targets and harvest goals accordingly. In these situations with a time-varying true Ricker *a* parameter, Kalman filter estimation resulted in greater mean cumulative catch than was produced by the standard linear regression approach, Walters' bias correction method, or a fixed harvest rate policy. This benefit of the Kalman filter resulted from its better ability to track changing parameter values, thereby producing escapements closer to the optimal escapement each year. However, errors in implementing desired management actions can significantly reduce benefits from all parameter estimation techniques.

Résumé : La productivité des populations de saumons du Pacifique peut connaître des changements persistants qui pourraient être liés aux changements climatiques. Les organismes de gestion doivent détecter avec rapidité et fiabilité ces changements pour éviter les pertes économiques pouvant découler de récoltes sous-optimales ou de la déplétion des stocks. Cependant, vu la variabilité inhérente des populations de saumon, il est difficile de détecter rapidement les changements, et encore plus de les prévoir. Nous comparons ici trois méthodes pour la mise à jour annuelle des estimations des paramètres entrant en jeu dans la relation stock-recrutement : régression linéaire ordinaire, régression avec correction de biais de Walter, et filtre de Kalman. Nous avons utilisé des simulations de Monte-Carlo fondées sur une vaste gamme de scénarios de changements dans le paramètre *a* de Ricker induits par le climat dans un stock de saumon. Nous avons ensuite appliqué chaque méthode d'estimation des paramètres aux données simulées sur le stock et le recrutement, puis établi des objectifs d'échappée et de capture en conséquence. Dans les situations avec un paramètre *a* vrai de Ricker variant dans le temps, l'estimation avec le filtre de Kalman a donné des captures cumulatives moyennes supérieures à celles données par la régression linéaire ordinaire, la méthode avec correction de biais de Walter, ou une politique imposant un taux fixe de capture. Cette supériorité du filtre de Kalman lui vient de sa plus grande capacité de suivre les valeurs changeantes des paramètres, ce qui lui permet de donner des échappées plus rapprochées de l'échappée optimale chaque année. Cependant, les erreurs dans la mise en oeuvre des mesures de gestion désirées peuvent réduire significativement les avantages de toutes les techniques d'estimation des paramètres.

[Traduit par la Rédaction]

Introduction

Fisheries scientists who work on Pacific salmon (*Oncorhynchus* spp.) traditionally assume that the parameters of the Ricker (1975) stock–recruitment relationship for a given stock (referred to here as productivity parameters) are constant over time and that the large observed year-to-year variability around that relationship is caused by environmental and other sources of variation. They then base management strategies, such as goals for spawner abundances, on estimates of those parameters as well as on other factors. However, this assumption of constant underlying mean parameters of stock–recruitment relationships may create serious management problems because there is now evidence that these parameters can shift dramatically over time.

For instance, in the 1970s, the *a* parameter of the Ricker stock–recruitment model rapidly increased in eight of nine major sockeye salmon (*Oncorhynchus nerka*) stocks from Bristol Bay, Alaska (Adkison et al. 1996). These increases in Ricker *a* parameters reflected the large and persistent increases in average survival rate from spawners to recruits (at any given abundance of spawners) that were observed after

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the early to mid-1970s in those stocks (Peterman et al. 1998), which also led to increased catches (Hare and Francis 1995). Adkison et al. (1996) attributed this shift in productivity parameters to climate-driven changes in oceanographic processes because there is strong evidence that a major change occurred in the mid-1970s in the physical and biological conditions of the Northeast Pacific Ocean (e.g., McLain 1984; Graham 1994), where most sockeye salmon rear for 2 or 3 years. One viable hypothesis is that the winter Aleutian low pressure atmospheric system and its associated winds intensified, as did upwelling, making the region more productive biologically: abundance of phytoplankton, zooplankton, and squid increased during that period (Venrick et al. 1987; Brodeur and Ware 1992).

Such shifts in productivity parameters have important implications for salmon management strategies. If stocks become more or less productive in the future, escapement goals (desired spawner abundance) and harvest rates should be adjusted accordingly. However, given the natural variability and measurement error in estimates of abundance of spawners and recruits, it has been difficult to detect a major signal (like the increase in productivity in Bristol Bay stocks) amidst the noise in stock–recruitment relationships until well after the event. This difficulty is demonstrated by the numerous papers published in the late 1980s and 1990s concerning the 1970s increase in productivity of Bristol Bay sockeye salmon, at least 10–15 years after the increase occurred. Clearly, such a lag leads to management actions that are out of step with the biological changes. To make matters worse, such changes in salmon productivity may actually be more common than previously thought because physical oceanographic systems can exhibit multiple stable states (or equilibria) due to the complex array of nonlinear lagged processes that create oceanic dynamics (e.g., Bryan 1986). In addition, major changes in freshwater and ocean systems may be occurring now, or may occur in the future, due to climatic change (e.g., Beamish 1995). Unfortunately, not only is it difficult to detect major changes in salmon productivity as they occur, but it is almost impossible to forecast them because we only qualitatively understand the complex mechanisms that link physical and biological oceanographic processes to salmon survival rates. It is thus important to continue research into the mechanisms of variability, but in the meantime, it is also critical to investigate improved ways of estimating parameters of salmon productivity so that we can better track the changes as they occur and revise management regulations quickly.

Therefore, our purpose was to compare various methods for estimating stock–recruitment parameters of salmon stocks in order to provide more timely indications of major changes in the values of those parameters. In particular, we focused on changes in parameters that could occur rapidly to some new level and then persist there for some time or changes that could occur more slowly but might be difficult to detect amidst variation (generally called time-varying parameters here). We used a simulation model of salmon populations and specified particular future hypothetical temporal trends in Ricker productivity parameters (scenarios). The model compared how well three different parameter estimation methods tracked such trends, as well as catches that would result from using those methods. Management implications of time-varying fish productivity parameters (also called nonstationarity by some authors) have only rarely been quantitatively investigated (e.g., Moussalli and Hilborn 1986; Walters 1986, 1987; Parma 1990; Parma and Deriso 1990; Walters and Parma 1996), and none of these researchers compared the ability of different statistical methods to accurately estimate changes in productivity parameters. Instead, they mainly focused on comparing different harvest policies in the presence of time-varying parameters.

Our focus on comparing methods for parameter estimation led us to explore the performance of the Kalman filter (Meinhold and Singpurwalla 1983), which is a type of time series model that was originally developed by control engineers. Its "prime objective is to estimate the signal in the presence of noise" (Chatfield 1989), which is exactly the situation facing fisheries scientists who try to estimate changes in productivity parameters. A Kalman filter differs from more common parameter estimation methods in fisheries in that it explicitly models not only observation error but also systematic temporal variation in parameters (Meinhold and Singpurwalla 1983). The Kalman filter has only been applied to fisheries problems by a few researchers (e.g., Walters 1986; Sullivan 1992; Pella 1993; Schnute 1994; Reed and Simons 1996; Newman 1998), and they did not apply it to the types of situations explored here.

Methods

General overview

We used a Monte Carlo simulation framework (Fig. 1) to evaluate three methods of annually updating parameter estimates of the Ricker stock–recruitment relationship: (*i*) standard linear regression, (*ii*) Walters' (1990) bias-corrected linear regression, and (*iii*) a Kalman filter. In each of 500 Monte Carlo trials (the number necessary to generate stable results), we simulated a hypothetical 100-year-long scenario of climate-induced change in productivity of a salmon stock. As discussed below, we represented such changes by a time-varying a parameter in the Ricker model that followed one of three functions: a sine wave, a step function, or an autoregressive process. For comparison, we also simulated the case of a constant a parameter. We then applied each estimation method to the simulated stock–recruitment data to annually estimate the Ricker parameters, which were in turn used to estimate optimal escapement targets and harvests. We compared parameter estimation methods in terms of cumulative harvests as well as various error statistics. We also compared these methods with fixed harvest rate policies and with scenarios where managers had perfect knowledge of the true Ricker parameters in the current year (i.e., the expected value of perfect information).

Stock–recruitment relationship

We simulated a wide range of hypothetical scenarios for climate-induced changes in salmon productivity because there is considerable uncertainty over how that productivity will change in the future. Specifically, to simulate the "true" salmon population dynamics, we used the Ricker model with a time-varying a parameter and a constant b parameter:

$$(1) \qquad \log_e(R_{t+m}/S_t) = a_t - bS_t + v_t$$

where R_{t+m} is total recruitment of age m adults produced from spawners, S, that spawned in year t and v is the i.i.d. residual variation with $v_t \sim N(0, \sigma_v^2)$. In the baseline case, we simulated a pink salmon (*Oncorhynchus gorbuscha*) population where all recruits

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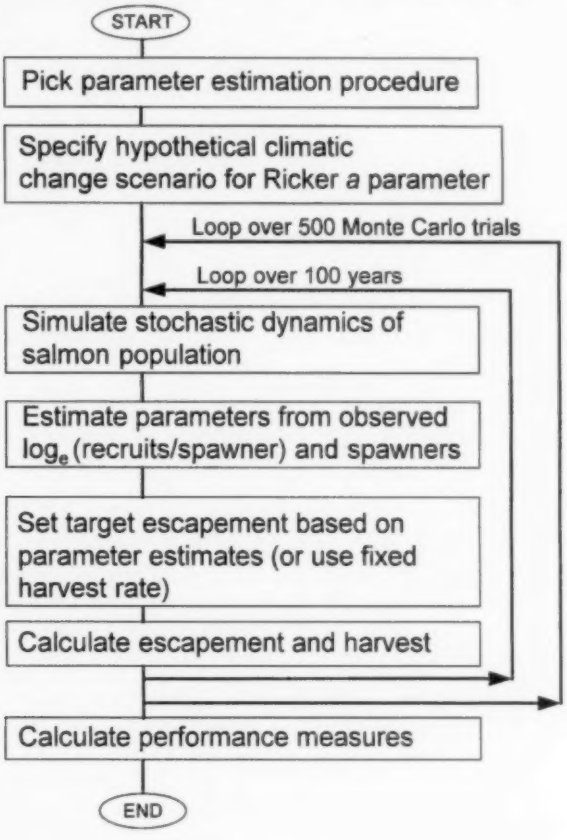
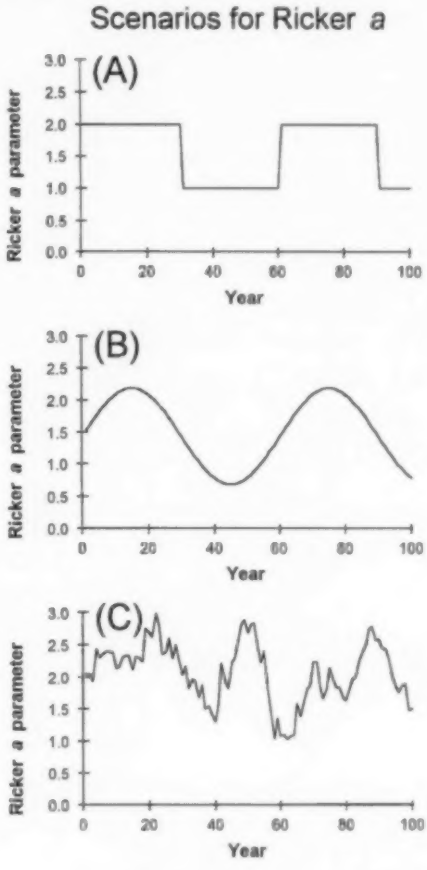
Fig. 1. Flowchart of the simulation model used to determine the relative merits of different methods for estimating Ricker stock–recruitment parameters when the *a* parameter varies over time.


START
Pick parameter estimation procedure
Specify hypothetical climatic change scenario for Ricker a parameter
Loop over 500 Monte Carlo trials
Loop over 100 years
Simulate stochastic dynamics of salmon population
Estimate parameters from observed log$_e$(recruits/spawner) and spawners
Set target escapement based on parameter estimates (or use fixed harvest rate)
Calculate escapement and harvest
Calculate performance measures
END

matured 2 years after they were spawned. The Ricker *a* parameter varied with time according to scenarios specified below. Unless stated otherwise, across all years and simulations, *b* was 1 (representing an arbitrary scalar for density dependence) and σ_v was 0.5, which resulted in variation typical for sockeye salmon (see below).

There are several reasons why we made the Ricker *a* parameter time varying, rather than the *b* parameter. First, as noted above, Adkison et al. (1996) showed that for eight of nine Bristol Bay sockeye stocks analyzed, there was an abrupt doubling (or more) of the *a* parameter in the Ricker model after the mid-1970s compared with before it (with a median factor of increase of 2.61 for the nine stocks), but there was no consistent change in the *b* parameter. Second, if the Ricker *b* parameter was time varying rather than *a*, it would imply that significant changes in survival rates (*R*/*S*) would occur only when spawner abundance (*S*) was large (i.e., a change in the level of density-dependent feedback). However, a changing *a* parameter reflects the more likely situation that climatic processes influence survival rates of a stock to a similar proportional degree regardless of the spawner abundance. Last, salmon researchers have known for many years that residuals from Ricker stock–recruitment curves are often autocorrelated, indicating slow changes in productivity over time (Hilborn and Walters 1992). In most past research, this has been modelled using an autocorrelated noise term, *v*, but as noted by Hilborn and Walters (1992), this is equivalent to assuming that the Ricker *a* parameter follows an autocorrelated temporal pattern.

Fig. 2. Examples of the three categories of time-varying Ricker *a* parameter explored: (A) step function, (B) sine wave, and (C) autoregressive (AR(1)) process. Figure 2C is only one example of autoregressive time trajectories generated by the 500 Monte Carlo trials.



Thus, our approach was to explicitly model the *a* parameter as an autocorrelated time series, but with additional random variation *v* to represent year-to-year changes in productivity unrelated to the slowly changing time series of *a* values. The scenarios for *a* were chosen to qualitatively reflect observed patterns in the productivity of fish stocks and oceanographic processes that are thought to influence stock productivity (sea surface temperature, atmospheric pressure, etc.). Although these time series for *a* were highly autocorrelated, the resulting data series of $\log_e(R/S)$ were less autocorrelated due to the influence of *v*. For example, in each scenario specified below except the constant *a* case, the lag-1 autocorrelation in simulated $\log_e(R/S)$ was roughly 0.6. This level of autocorrelation is consistent with that observed in Bristol Bay sockeye data sets (Korman et al. 1995). Variations on the baseline scenarios below are described in the section Sensitivity analyses.

Scenarios for changes in salmon productivity

The "step function" scenario simulated abrupt increases or decreases (Fig. 2A) in productivity that persisted for a specified number of years. As noted above, there is evidence for this type of abrupt change in productivity for Alaska sockeye (Adkison et al. 1996; Peterman et al. 1998) and pink salmon (Hare and Francis

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1995). Because these stocks appeared to become unusually productive in the 1970s, and because there is concern that they may return to a less productive regime in the future (if they have not already done so), we simulated an initial step down, with the a parameter decreasing from 2.0 to 1.0. The only data available for determining the duration of such relatively productive and unproductive intervals are catch data back to 1925 in Hare and Francis (1995). Although catch data do not measure productivity as directly as survival rates, they do provide limited evidence that such intervals last about 30 years, giving a full cycle every 60 years. We used that 60-year period in lieu of any other evidence.

The "sinusoidal" scenario used a sine wave in Ricker a (Fig. 2B) to simulate cyclical, but more gradual, decadal-scale variations in productivity:

$$(2) \qquad a_t = a_0 + A \sin\left(\frac{2\Pi}{Pt}\right)$$

where a_0 is the initial a value, A is the amplitude, and P is the period of the sine wave in years. Baseline values were $a_0 = 1.5$, $A = 0.75$ (giving a peak-to-trough range in a from 2.25 to 0.75), and $P = 60$ years.

In contrast with the deterministic step and sine wave functions, we also simulated time series of Ricker a as a stochastic process using a first-order autoregressive (AR(1)) model:

$$(3) \qquad a_t = a_0 + z_t$$

where

$$(4) \qquad z_t = \phi z_{t-1} + \varepsilon_t.$$

Here, a_0 is the mean of a_t, z_t is an AR(1) process with mean zero, ϕ is the lag-1 autocorrelation, and ε is an i.i.d. variable with $\varepsilon_t \sim N(0, \sigma_\varepsilon^2)$. The variance of a is (Box and Jenkins 1976)

$$(5) \qquad \sigma_a^2 = \sigma_z^2 = \frac{\sigma_\varepsilon^2}{(1-\phi^2)}.$$

Baseline values were $a_0 = 1.5$, $\phi = 0.9$, and $\sigma_\varepsilon = 0.218$ (i.e., σ_ε calculated as a function of ϕ and $\sigma_a = 0.5$ by rearranging eq. 5). Figure 2C shows one example of this "autoregressive" scenario.

For comparison, we also simulated a case in which the Ricker a parameter was constant at 1.5 across all years. We did this to reflect the common assumption that parameters of salmon stock–recruitment curves remain fixed over time. For this scenario, σ_v in eq. 1 was set to 0.7. This generated similar variance in $\log_e(R/S)$ as the previous scenarios, in which part of the variation arose from time-varying a parameters.

Thus, true Ricker a values were the same for a given t in each Monte Carlo trial for a given step, sine wave, or constant a scenario but differed among trials for the autoregressive case. However, each trial in all scenarios for the Ricker a parameter included the random variation term, v_t, in eq. 1.

Parameter estimation methods

Standard linear regression and Walters' (1990) time series bias correction

Our first estimation procedure was Ricker's (1975) standard linear regression of $\log_e(R/S)$ on S, formulated as in eq. 1 to provide annual estimates of a, b, and σ_v via least squares minimization. As in all parameter estimation methods explored here, the baseline regression case used all available stock–recruitment data up through year t. However, in the sensitivity analysis of this standard regression approach, we also evaluated whether using a smaller "window" of data points would allow for better tracking of changes in productivity and improved management performance because omitting older data might improve annual estimates of a and b when the true a changes rapidly. Thus, in separate analyses, we explored 17 different widths of windows (e.g., only the most recent 5 years, 10 years,..., and up to 85 years of data).

The second estimation procedure was Walters' (1990) time series bias correction to the standard linear regression approach. This correction attempts to reduce biases in the standard least squares estimates of Ricker a and b that occur because of a lack of independence between S and the dependent variable $\log_e(R/S)$. Again, this approach used stock–recruitment data for all years available up to a given time t except in the sensitivity analysis, where we also explored smaller recent windows of data.

Thus, the standard linear regression and Walters' bias correction method reflected the common assumption made by salmon fisheries scientists that the Ricker a and b parameters are constant through time, although these methods updated their parameter estimates annually.

Kalman filter

The third parameter estimation method, a Kalman filter, is designed to help estimate an underlying time-varying signal amidst noise. Clear introductions to this technique are given in Meinhold and Singpurwalla (1983) and Chatfield (1989).

In the state–space model formulation of the Kalman filter, there are two components: the "observation equation" and the "system equation." The observation equation describes the relationship between observed quantities, such as recruits and spawners. We used the linear form of the Ricker model (eq. 1) for the observation equation, where v is defined in the Kalman filter method as "observation error." In contrast, the system equation describes the dynamic process of change of model parameters through time. The parameter estimation routine of course does not know the temporal pattern of change in the true Ricker a parameter (step function, sine wave, etc.), so as is commonly done in such situations, we defined the system equation as a random walk to provide a wide range of flexibility for tracking shifts in productivity:

$$(6) \qquad a_t = a_{t-1} + w_t$$

where $w_t \sim N(0, \sigma_w^2)$ is the "system error." For reasons noted above, we assumed that the Ricker b parameter was not a dynamic component of the underlying model.

Details of the Kalman filter calculations are provided in the Appendix. In brief, this procedure involves recursively estimating a each year based on the previous year's estimate of a and the new observation of $\log_e(R/S)$. In addition, estimates of the other fixed model parameters (b, σ_v, and σ_w) are annually updated using maximum likelihood based on all past data. The Kalman filter assumes a time-varying intercept, a_t, and that $\sigma_w^2 > 0$. A key difference from the standard constant-parameter linear regression approach (which assumes $\sigma_w^2 = 0$) is that the Kalman filter revises parameter estimates faster; it "gives more weight to the most recent forecast error" (Abraham and Ledolter 1983, p. 367).

Population dynamics and management regulations

For each 100-year Monte Carlo trial, we initialized spawner abundances for the first m years (where m is age-at-maturity of adults) using a uniform random distribution between 0.2 and 0.8 (arbitrary units). The model then used eq. 1 to stochastically generate 10 years of "historical" spawner–recruit data using a fixed annual harvest rate of 50%, which generated a reasonable contrast in abundance. These historical data avoided problems that would have been created by routines attempting to estimate model parameters and target escapements with very few data points early in simulations.

In each of the remaining 90 years, Ricker parameters were estimated using the method being examined, based on observed spawner–recruit data up through the previous ($t - 1$) year's returns.

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To reflect the response of managers, these parameter estimates were then used to set the target escapement and hence harvest strategy in year t. This created a 1-year lag between when parameter estimates were made and when they were used to set the harvest strategy. Note, however, that because of the 2-year life cycle of pink salmon, the Ricker parameters estimated from a given year's recruitment (say in 1999) would estimate the productivity of fish that were spawned in 1997. Hence, because these estimates would only be available to set management targets the next year (2000), there was an inevitable 3-year lag in estimates of Ricker a and b behind their true values in nature.

Specifically, we used the most recent estimates of Ricker parameters ($\hat{a}$, $\hat{b}$, and $\hat{\sigma}_v$) to estimate the "optimal" or target escapement, S_{target}, each year, i.e., the escapement that would maximize the excess of recruits over spawners. To do this, we fit a four-parameter, inverse S-shaped function (a modified Michaelis–Menten equation) to the relationship between optimal escapement and the Ricker a parameter (Hilborn 1985). Our function was more accurate over a wider range of a values than Hilborn's (1985) two-parameter function:

$$(7) \qquad S_{\text{target}} = \frac{a'}{\hat{b}}\left(K_1 - \frac{K_2 a'^{K_3}}{K_4 + a'^{K_3}}\right)$$

where the Ricker a parameter was adjusted to account for lognormal variation (i.e., $a' = \hat{a} + (\hat{\sigma}_v^2/2)$; Hilborn 1985) and where $K_1 = 0.5$, $K_2 = 0.65$, $K_3 = 8.7$, and $K_4 = 1.27$.

When recruits exceeded the target escapement, catch was simply recruits minus S_{target}. However, if recruitment was below S_{target}, the harvest was set to zero and all recruits became spawners. Under some conditions of very short data sets or extreme outliers in stochastically generated data points, Ricker parameter estimates fell outside the acceptable range (i.e., $0 < a < 4$ or $b < 0$). In such cases, we instead used a conservative harvest rate of 20% and let 80% of fish escape. This situation only occurred infrequently, except for Walters' bias correction method, where it occurred as often as 20% of the time.

For each scenario of climatic change, we also explored a range of fixed harvest rates (5–95%), as suggested by Walters and Parma (1996), to see how this policy performed compared with policies in which target escapements were set annually as a function of current estimates of productivity parameters. The best fixed harvest rate was the one that maximized long-term cumulative catch.

Expected value of perfect information

To put the performance of our parameter estimation methods into some context, we compared the simulated catches with the catch that would have been obtained if managers had perfect information about the Ricker parameters each year. We thus determined the expected value of perfect information by conducting simulations using "true optimal" escapements, i.e., where target escapements (eq. 7) were based on the true values of the Ricker parameters in the current year t. This closely resembled the "myopic" policy simulated by Walters and Parma (1996). It assumes that the manager knows what productivity conditions this year's spawners will experience but does not assume knowledge about the full time trajectory of future productivities, as did Parma (1990) and Walters and Parma (1996) in their "perfect information" policies.

Performance measures

To compare parameter estimation routines and harvest policies, we calculated two classes of performance measures: management and statistical. Performance measures were calculated across the 90 years following the initial 10 years of historical data and were then averaged across all 500 Monte Carlo trials.

Management performance measures were the key measures used for comparing alternative harvest policies and parameter estimation procedures. In particular, maximizing cumulative catch (i.e., sum of catches over all 90 years) was the main criterion for ranking. We also recorded the coefficient of variation (CV) in catch as a measure of year-to-year variability and calculated several measures of spawning stock abundance, including average abundance, CV in abundance, and proportion of years in which spawner abundance was below an arbitrary conservation threshold (7% of the average unfished equilibrium abundance).

To understand why some parameter estimation techniques generated greater cumulative catches than others, we calculated statistical performance measures that compared the true Ricker parameters with parameter values generated by the estimation routines. The resulting error term in each year was E_t, defined as the estimated parameter value used to set the escapement target in that year minus the true value applied to offspring of fish that spawned that year. To indicate accuracy, we calculated the mean percent bias (%MB) of Ricker a estimates, for example, as

$$(8) \qquad \%\text{MB} = \frac{\sum_{t=1}^{n}\left(\frac{E_t \cdot 100}{a_t}\right)}{n}$$

where n is the number of years of data. We also calculated the mean squared error (MSE) of parameter estimates:

$$(9) \qquad \text{MSE} = \frac{\sum_{t=1}^{n} E_t^2}{n}.$$

Analogous mean percent bias and MSE statistics were calculated for actual escapement compared with the true optimal escapement.

Sensitivity analyses

We varied some assumptions from baseline runs to determine the robustness of our conclusions. For instance, we explored variations on the scenarios for changes in the Ricker a parameter. First, the step function and sine wave runs were changed to have a 30-year cycle, rather than 60 years. Second, we changed the autoregressive a runs to have a lag-1 autocorrelation of 0.7 instead of the baseline 0.9. Third, for each baseline a-change scenario, we explored values of σ_v (random error term in eq. 1) of 0.3 and 0.7 rather than the baseline value of 0.5. Fourth, we also simulated a sockeye salmon population with a fixed 4-year life cycle (e.g., a Fraser River, British Columbia, stock). Last, we incorporated "implementation error" (Rosenberg and Brault 1993) into our management subroutine to more closely approximate the imperfect control that managers have over implementing harvest policies. In baseline runs, escapement targets were met exactly (i.e., perfect harvest control) if recruits in a given year were greater than the target abundance of spawners. However, in practice, there is incomplete control over harvesters and actual spawner abundance can deviate from the target, in some cases by a considerable amount. Generally, when there is a high abundance of recruits, spawner abundance ends up greater than the target, and conversely, when recruitment is low, the target is not usually reached (e.g., Bocking and Peterman 1988; Robb and Peterman 1998). This pattern of under- and over-escapement results from the common interaction between errors in preseason forecasts and the lag in in-season updates of those forecasts. By the time a large recruitment is confirmed, for example, it is often too late to harvest all fish in excess of the target escapement. Another source of implementation error is the tendency by managers to avoid extreme changes in fishing time from one year to the next.

Therefore, we incorporated implementation error into sensitivity analyses using

$$(10) \qquad S_t = S_{\text{target}}\,[c + (dR_t)](1 + \text{errdev})$$

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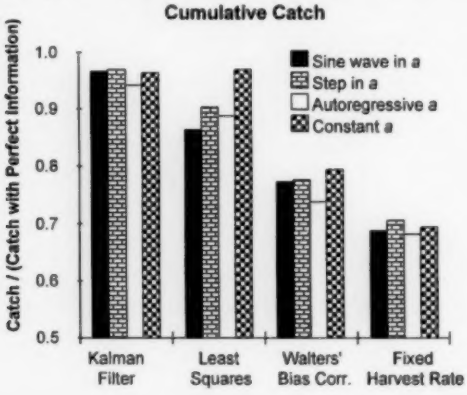
Fig. 3. Expected cumulative catch during 100-year simulations (averaged over 500 Monte Carlo trials) resulting from using different methods for estimating Ricker parameters (Kalman filter, standard least squares, or Walters' bias correction method) and by using the best fixed harvest rate. Results are shown for each baseline scenario for the time-varying Ricker *a* parameter as well as the constant *a* scenario. The *y*-axis is in units of expected catch as a proportion of the expected catch that would result from having perfect information about true parameter values.



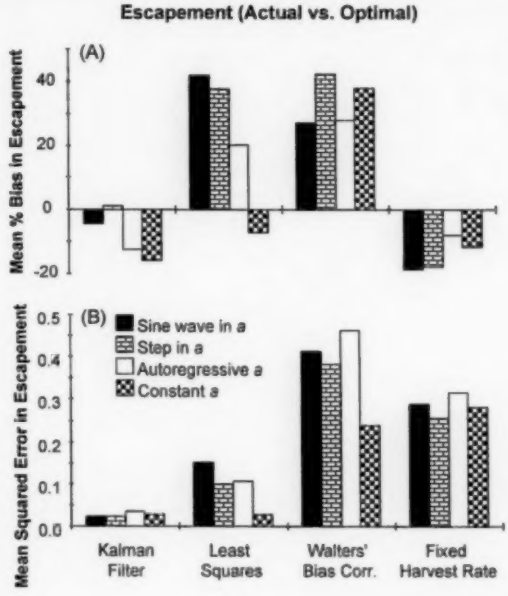
to relate the actual escapement in a given year (S_t) to both the target escapement (S_{target}) and recruits that year (R_t). We simulated two possible cases of implementation error. First, we imposed both bias and imprecision in a way that reflected the situation described in the previous paragraph by setting $c = 0.5$, $d = 0.3$, and "errdev" to be normally distributed with mean 0 and $\sigma = 0.2$. In a more optimistic case for a separate sensitivity run, we assumed that implementation error was only imprecise and not biased. That is, we imposed only normally distributed random variation (with $\sigma = 0.2$) around the target escapement (i.e., $c = 1.0$, $d = 0$).

Results

In scenarios where the true Ricker *a* parameter was assumed to vary with time, estimating parameters by using the Kalman filter resulted in greater mean cumulative catch over the 100-year simulations than catch produced by either the standard least squares approach or Walters' (1990) bias correction method. When compared with the next-best method (least squares), the benefit of the Kalman filter was 12.2% greater expected catch for an underlying 60-year sine wave in *a*, 7.2% greater catch for a 60-year step function, and 6.1% greater for an autoregressive *a* over time (Fig. 3). However, for the scenario with a constant *a*, these two methods gave roughly the same expected catch. Walters' bias correction method consistently performed more poorly than both the Kalman filter and the least squares methods. All three of these methods for annually updating Ricker parameters and target escapements generated greater expected catch than the best fixed harvest rate strategy (Fig. 3). In fact, the Kalman filter produced about 40% greater catch than the best fixed harvest rate strategy in all four baseline scenarios (Fig. 3).

These catch results can be explained by examining how well the actual annual escapements of spawners approximated the true optimal escapements (as defined by the true

Fig. 4. (A) Mean percent bias in actual escapement (compared with the optimal escapement if the true Ricker parameters were known each year) resulting from using different methods for estimating Ricker parameters and from using the best fixed harvest rate. (B) Same as Fig. 4A, except the *y*-axis represents MSE in escapement (actual compared with optimal escapement).



Ricker *a*, *b*, and σ_v parameter values). In all scenarios except constant *a*, actual escapements for Kalman filter runs were much less biased (when compared with the true optimal escapement) than for the least squares or Walters' methods; the latter two methods tended to overestimate the time-varying optimal escapement by 20–43% (Fig. 4A). Furthermore, actual escapements for the Kalman filter also approximated the true optimal escapements much more consistently (i.e., lower MSE) (Fig. 4B). This closer attainment of the optimal escapement reduced the frequency of suboptimal escapements (either too high or too low), which increased future recruitment and resulted in higher cumulative catches over the 90-year simulations (e.g., Fig. 5). Note that although Walters' method resulted in smaller bias than least squares for the sine wave case (Fig. 4A), it had much greater MSE in actual escapement than either least squares or the Kalman filter (Fig. 4B), thus also leading to frequent over- or under-escapement. This wide range of realized escapements also occurred for the best fixed harvest rate (e.g., 65% in the case of the 60-year sine wave). In fact, across all simulations with a time-varying *a* parameter (including sensitivity analyses reported below except implementation error), the CVs in spawner abundance for Walters' method (0.64–0.79) and the fixed harvest rate strategy (0.96–1.06) were at least three times larger than those for the Kalman filter (0.21–0.25). Consequently, the Kalman filter produced at least 21% greater catch than Walters' method (18% greater for the implementation error case shown below) and at least 37% greater catch than the best fixed harvest rate. There-

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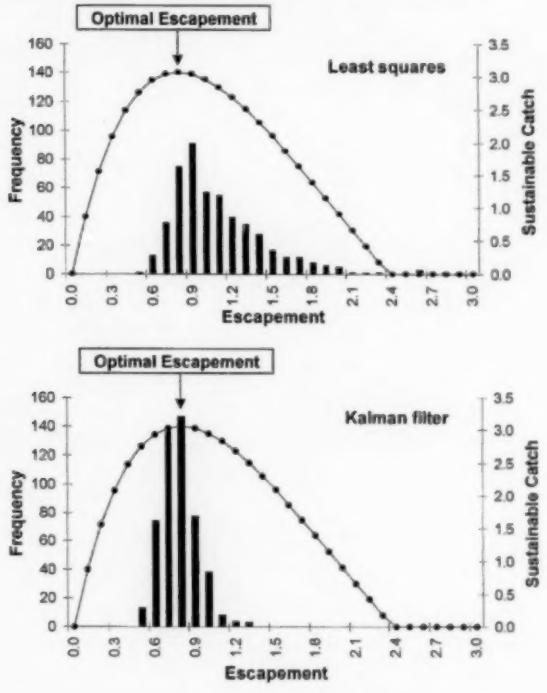
Fig. 5. Frequency distribution of escapements (bars) resulting from using either least squares (top panel) or the Kalman filter (bottom panel) to estimate Ricker parameters compared with the optimal escapement based on the true parameters. Each distribution shows the escapements realized in the 500 Monte Carlo trials for the baseline sine wave case at the arbitrarily chosen year 75. In that year, the true Ricker a parameter = 2.25 and true b = 1. Curves indicate the annual sustainable catch that would result from a given escapement for those true parameter values.

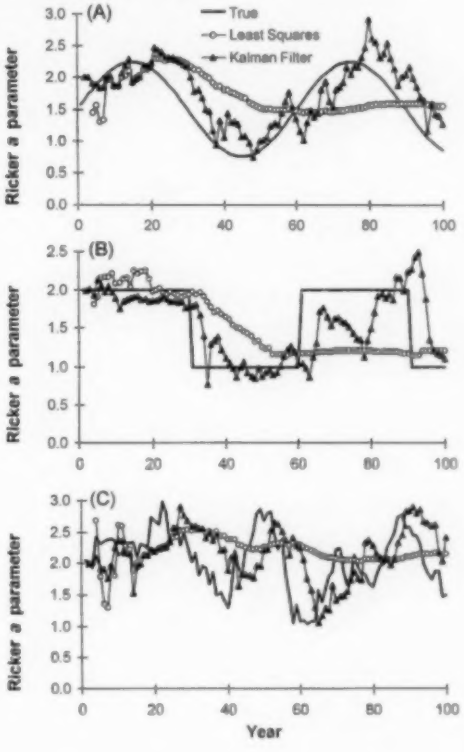


fore, we do not present further results for Walters' bias correction or fixed harvest rate methods.

These differences in performance among parameter estimation methods resulted from their different assumptions about the constancy of Ricker parameters and therefore different abilities to accurately estimate these parameters under the various scenarios of a changing a parameter. The least squares approach assumed constant parameters, and after a few decades of data had accumulated, its parameter estimates were dominated by the many data points arising in earlier years. It therefore tended to track changes poorly in comparison with the Kalman filter (e.g., Fig. 6). Over each set of 500 Monte Carlo trials, this behavior resulted in parameter estimates for a and b that had lower MSE for the Kalman filter than for least squares (Fig. 7B). This lower MSE for the Kalman filter apparently more than offset the effect of greater bias that it showed compared with least squares (Fig. 7A), as illustrated by the greater catches for the Kalman filter reported above (Fig. 3).

The poor tracking of least squares suggests that some "discounting" of past data (Walters 1986) might be more appropriate and could allow the regression estimates to respond better to changes in true parameter values that occur

Fig. 6. One example Monte Carlo trial for each of the baseline scenarios for the Ricker a parameter: (A) 60-year sine wave, (B) 60-year step function, and (C) autoregressive (AR(1)) process. Annual true Ricker a values (line without symbols) were the same in each Monte Carlo trial for the sine wave and step cases but differed in each trial for the autoregressive case. However, the Ricker a values estimated by least squares (circles) and a Kalman filter (triangles) differed in each Monte Carlo trial for all three scenarios shown here, as well as for the case of a constant a parameter. Although they were not used in years 1–10 to calculate performance measures or to set target escapements, parameter estimates are shown here for those years to illustrate the relative instability caused by few data points.



later in the 100-year period. However, shorter windows for including data in the regression (e.g., the most recent 5 years, the most recent 10 years,..., up to 85 years) did not result in greater cumulative catch than using all previous data. Using shorter sets of recent data resulted in more variable parameter estimates, which resulted in higher frequencies of suboptimal escapements and reduced catches. Therefore, all least squares results presented here came from that method's best case, the baseline, which included all data up to the year of parameter estimation.

Sensitivity analyses

Our baseline conclusion, that greater catch would be expected from using the Kalman filter, was robust to several changes in assumptions. For instance, the Kalman filter again gave greater catch than least squares (16.3% greater

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Fig. 7. (A) Mean percent bias in estimates of Ricker *a* and *b* parameters (compared with their true values each year) resulting from using either the Kalman filter or the least squares method. (B) Same as Fig. 7A, except the *y*-axis represents MSE in parameter estimates.

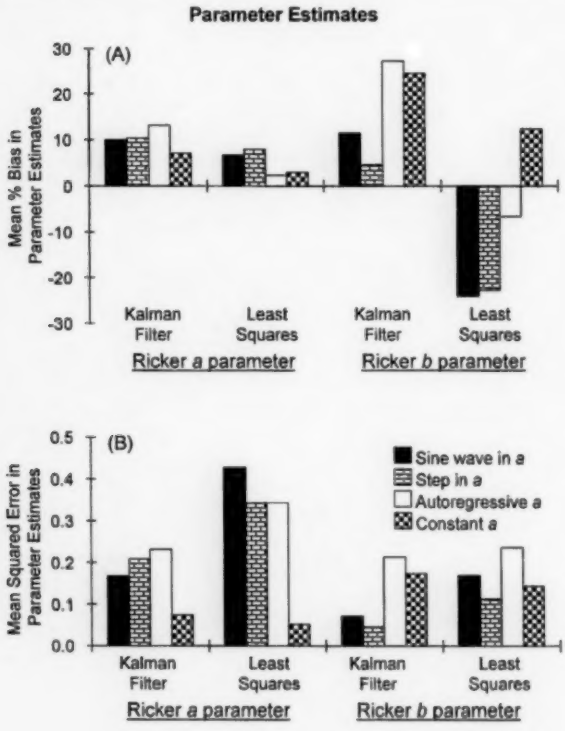
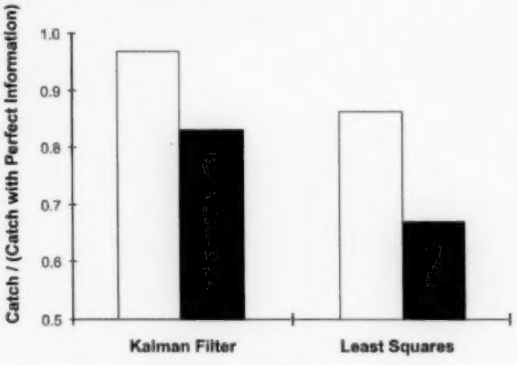


Fig. 8. Effect of the first type of implementation error (which included both bias and imprecision) on expected cumulative catch during 100-year simulations (averaged over 500 Monte Carlo trials). Catches resulted from using either the Kalman filter or the standard least squares to estimate Ricker parameters. Results are shown for the baseline sine wave scenario with (solid bars) and without (open bars) imposing error in implementing the estimated harvest goal. The *y*-axis is expected catch as a proportion of the expected catch that would result from having perfect information about true parameter values.



for the sine wave and 7.4% for the step function) when the sine wave and step function runs had 30-year rather than 60-year cycles. Thus, even if future changes were to occur relatively rapidly compared with the past century, the Kalman filter would likely be better than least squares at tracking those changes. However, in general, as the magnitude or rate of change in the sine wave or step case was reduced, so too was the difference between the catch from the Kalman filter and least squares (i.e., they approached the constant *a* case). Similarly, when the autoregressive *a* runs used a lag-1 autocorrelation of 0.7 instead of the baseline 0.9, the Kalman filter catch was only 0.5% greater than that for least squares. Results were also sensitive to the value of the random error term σ_v. For example, for the sine wave scenario, the Kalman filter gave 29.6% greater catch than least squares when $\sigma_v = 0.3$ (compared with 12.2% when $\sigma_v = 0.5$) but only 5.6% when $\sigma_v = 0.7$. Across all *a*-change scenarios, the difference between Kalman filter and least squares catch generally declined as σ_v increased because this greater random variation reduced the Kalman filter's ability to track changes in *a*.

The Kalman filter also performed best in the other two sensitivity analyses. It gave 11.8% greater catch than the least squares method in the case of a sockeye salmon population with a fixed 4-year life cycle (compared with the baseline 2-year pink salmon case). Thus, the longer lag in obtaining information on the fate of each cohort of offspring did not change the rank order of parameter estimation methods. In addition, in all scenarios, results for the Kalman filter were also insensitive to the choice of initial values for the mean and variance of the Ricker *a* parameter, as long as reasonably large (i.e., uninformative) variances were used (see Appendix).

Results to this point were derived from analyses where the model assumed "perfect control," i.e., an escapement goal determined from the current parameter estimates was achieved exactly when recruitment was greater than that goal, and catch was simply the excess of recruitment over the goal. When recruitment was too low, catch was zero. This perfect control is one reason why average cumulative catches for the least squares and Kalman filter methods were within 15% of the catch that could be obtained if managers had perfect information about the true Ricker parameter values (Fig. 3). The other reason is that the Ricker model causes a relatively flat relationship between spawner abundance and sustainable catch near the optimum escapement (Fig. 5). However, when we instead included implementation error (Rosenberg and Brault 1993) to reflect the imperfect control that managers have over implementing harvest policies, all parameter estimation methods generated lower cumulative catch because target escapements were achieved less often. Furthermore, there were smaller differences among catches from different parameter estimation methods. However, the Kalman filter was still superior and its performance degraded less than least squares in the presence of implementation error. For instance, for the scenario with a sine wave in *a*, Kalman filter catch dropped by only 14% when we imposed our first type of implementation error, whereas it dropped by 22% for least squares (Fig. 8). In the more optimistic case for implementation error, where we assumed that it was only imprecise and not biased relative to

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the target escapement, the Kalman filter catch was still 11% greater than that for least squares.

Discussion

Our results suggest that there may be a distinct advantage, and very little disadvantage, in using a Kalman filter approach to annually estimate parameters of the Ricker stock–recruitment model and then to update escapement goals accordingly. Our results indicate that if changes in salmon productivity can be represented by temporal trends in Ricker *a* parameters (e.g., the step function for Bristol Bay, Alaska, sockeye salmon; Adkison et al. 1996), then future cumulative catch will be larger using Kalman filter estimation than the current widely applied least squares formulation that assumes constant Ricker parameters. A disadvantage in using the Kalman filter (and it is slight, only a 0.5% reduction in catch) occurs if the Ricker parameters for a stock are actually constant over time. However, this constant scenario seems unlikely, given the growing evidence that productivities of the ocean and salmon stocks change with time (Venrick et al. 1987; Brodeur and Ware 1992; Beamish 1995; Adkison et al. 1996; Mantua et al. 1997; Peterman et al. 1998). Therefore, we strongly recommend that salmon scientists consider using a parameter estimation method, such as the Kalman filter, that explicitly models time-varying productivity parameters and is flexible enough to track various types of temporal changes in those parameters. Furthermore, like Korman et al. (1995), we do not recommend Walters' bias-correction method because in our trials, it had larger MSE in its parameter estimates and hence generated poorer estimates of target escapement.

The current approach used by many salmon scientists, least squares estimation, will fail to reflect changes in true parameters and will eventually converge to an estimate in the middle of the range of variation (e.g., Fig. 6A). One alternative that we examined, which was to eliminate data older than some year and use least squares on the remaining data, created less reliable parameter estimates and consequently a higher frequency of suboptimal estimates of escapement targets.

The relatively good performance of the Kalman filter in the face of a wide variety of types of future scenarios in productivity parameters is important for fisheries managers because our understanding of the linkage among changes in physical oceanographic conditions, nutrients, primary production, secondary production, and salmon is too limited to make reliable forecasts of changes in salmon productivity. Managers will for some time continue to be unsure whether productivity for a given salmon stock will increase or decrease and how fast it will change, creating uncertainty about the appropriate spawning escapement. However, the Kalman filter appears to be an improvement upon current approaches for tracking such changes and estimating appropriate escapements, whatever trends in productivity might occur in the future. Furthermore, while our initial focus was on how to best detect climatically driven changes in oceanographic conditions affecting salmon, the Kalman filter is general enough that it would perform equally well if future changes in the Ricker *a* parameter resulted from alterations in survival rate during the freshwater life stage (e.g., Schaller et al. 1999).

We also found that a Kalman filter had considerably greater average cumulative catch (at least 37%) than the best fixed harvest rate strategy, yet Parma (1990) and Walters and Parma (1996) pointed out the advantage of the latter. Their conclusion about a fixed harvest rate strategy differs from ours partly because, for some of their analyses, they used a different management objective. We aimed to maximize cumulative catch, whereas Walters and Parma (1996) attempted to reduce the year-to-year variation in catch while maintaining reasonably high cumulative catch. They also noted the relative ease of implementation of a fixed harvest rate strategy in some fisheries (e.g., salmon). However, even when considering cumulative catch alone, we found that the disadvantage of using a fixed harvest strategy was much greater than the 10–15% reduction in cumulative catch estimated by Walters and Parma (1996). This difference was partly due to our use of additional random variation, σ_v (a process not considered by Walters and Parma (1996)), to simulate year-to-year changes in productivity unrelated to the slowly changing patterns in *a*. When we set $\sigma_v = 0$ in each *a* change scenario, the best fixed harvest rate produced catches that were roughly 82% of those obtained with perfect information, compared with just 70% when $\sigma_v = 0.5$ (Fig. 3). In addition, for most of their results, Walters and Parma (1996) assumed that the true unfished equilibrium varied over time. In contrast, we assumed that the density-independent Ricker *a* parameter varied over time, which may have also made a fixed harvest rate perform worse than they indicated. Moreover, for a given time-varying scenario for *a*, we found that the between-year variability (CV) in catch for the Kalman filter (1.01–1.06) was at most only 10% greater than that for the best fixed harvest rate strategy (0.96–1.06), and the Kalman filter also had at least a 10% lower CV in catch than least squares. Apparently, because the Kalman filter led to more frequent achievement of escapements close to the optimum, it generated a relatively steady, large catch.

None of the three methods for annually updating parameters raised conservation concerns because spawner abundances were rarely low (< 0.3% of all cases without implementation error). Even in the presence of implementation error, spawners were low in less than an average of 5% of the years for all parameter estimation techniques. In contrast, under the best fixed harvest rate strategy, this probability was as high as 26%.

While Walters (1987), Parma (1990), and Walters and Parma (1996) addressed situations with time-varying productivity of fish stocks, they focused on deriving optimal management strategies for given situations (e.g., whether to increase or decrease escapement when future improved productivity was anticipated and whether to actively experiment with harvest rates to generate information). In contrast, we focused mainly on comparing different methods for estimating the changing parameters of productivity. Furthermore, our results and those of Walters (1987), Parma (1990), and Walters and Parma (1996) are not directly comparable because they used different underlying models (e.g., delay-difference population model, different time-varying component from ours) and made more restrictive assumptions. For

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instance, depending on the example, Parma (1990) assumed either that the future time trajectory of productivity parameters is known at any time or that managers know the probability distribution function from which productivity parameters are drawn. In contrast, we assumed that managers knew the functional form of the true underlying stock–recruitment relationship (Ricker model) but did not know its parameter values, the future trajectory of parameters, or probability density functions of parameter values.

Other fisheries researchers have applied a Kalman filter (Walters 1986; Sullivan 1992; Pella 1993; Reed and Simons 1996; Newman 1998), but to different problems than ours. In general, these authors support further application of the Kalman filter approach and we concur.

Limitations

Our results may be contingent upon various assumptions. First, we used analyses of Bristol Bay, Alaska, sockeye salmon from the 1950s through the 1980s (Adkison et al. 1996) to justify our assumption that a time-varying Ricker *a* parameter is the best way to represent possible future changes in salmon productivity. However, it is conceivable that for other stocks or species, future changes might be better represented by a dynamic *b* parameter. The Kalman filter could be written to reflect that situation, but we have not yet evaluated its performance in this context because of the weight of current evidence supporting a dynamic *a* parameter. Second, we assumed an underlying Ricker stock–recruitment model for both the true population dynamics and the parameter estimation methods, as is assumed almost universally by Pacific salmon biologists. However, if another model more adequately describes the underlying dynamics in nature, including other forms of noise terms, then our results for all parameter estimation methods are too optimistic in terms of being able to estimate changes. Finally, the relative advantage of the Kalman filter over the least squares approach is affected by the magnitude, rate, duration, and to some extent the frequency of change in productivity. For instance, that advantage will be reduced when the "signal" is decreased by having only a subtle change in productivity. Despite these limitations, our results suggest a direction for future research to improve the ability of management agencies to track and respond to changes in underlying productivity of salmon stocks.

Management implications of implementation error

Our sensitivity analyses on implementation error, which causes actual escapement to deviate from the target for reasons other than errors in Ricker parameter estimates, emphasize the need for further advances in other areas of fish stock assessment and management. Specifically, the potential advantage of a Kalman filter over the standard least squares method in tracking changes in productivity could be drastically diminished (along with catch resulting from any estimation method) unless implementation error is reduced. A decrease in this error could result from a combination of better preseason forecasts of abundance, improved stock identification in mixed-stock fisheries, more timely and accurate in-season estimation of abundance, and greater compliance with regulations by harvesters.

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References

Abraham, B., and Ledolter, J. 1983. Statistical methods for forecasting. John Wiley & Sons, New York.

Adkison, M.D., Peterman, R.M., Lapointe, M.F., Gillis, D.M., and Korman, J. 1996. Alternative models of climatic effects on sockeye salmon productivity in Bristol Bay, Alaska, and the Fraser River, British Columbia. Fish. Oceanogr. **5**: 137–152.

Beamish, R.J. (*Editor*). 1995. Climatic change and northern fish populations. Can. Spec. Publ. Fish. Aquat. Sci. No. 121.

Bocking, R.C., and Peterman, R.M. 1988. Preseason forecasts of sockeye salmon (*Oncorhynchus nerka*): comparison of methods and economic considerations. Can. J. Fish. Aquat. Sci. **45**: 1346–1354.

Box, G.E.P., and Jenkins, G.W. 1976. Time series analysis: forecasting and control. Revised ed. Holden-Day, San Francisco, Calif.

Brodeur, R.D., and Ware, D.M. 1992. Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. Fish. Oceanogr. **1**: 32–38.

Bryan, F. 1986. High-latitude salinity effects and interhemispheric thermohaline circulations. Nature (Lond.), **323**: 301–304.

Chatfield, C. 1989. The analysis of time series. An introduction. Chapman and Hall, New York.

Graham, N.E. 1994. Decadal-scale climate variability in the tropical and North Pacific during the 1970s and 1980s: observations and model results. Clim. Dyn. **10**: 135–162.

Hare, S.R., and Francis, R.C. 1995. Climate change and salmon production in the Northeast Pacific Ocean. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 357–372.

Hilborn, R. 1985. Simplified calculation of optimum spawning stock size from Ricker's stock recruitment curve. Can. J. Fish. Aquat. Sci. **42**: 1833–1834.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Korman, J., Peterman, R.M., and Walters, C.J. 1995. Empirical and theoretical analyses of correction of time-series bias in stock–recruitment relationships of sockeye salmon. Can. J. Fish. Aquat. Sci. **52**: 2174–2189.

Mantua, N.J., Hare, S.R., Zhang, Y., Wallace, J.M., and Francis, R.C. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bull. Am. Meteorol. Soc. **78**: 1069–1079.

McLain, D.R. 1984. Coastal ocean warming in the northeast Pacific, 1976–83. *In* The influence of ocean conditions on the production of salmonids in the north Pacific. *Edited by* W.G. Pearcy. Oreg. State Univ. Sea Grant College Progrm ORESU-W-83-001. pp. 61–86.

Meinhold, R.J., and Singpurwalla, N.D. 1983. Understanding the Kalman filter. Am. Stat. **37**: 124–127.

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Moussalli, E., and Hilborn, R. 1986. Optimal stock size and harvest rate in multistage life history models. Can. J. Fish. Aquat. Sci. **43**: 135–141.

Newman, K.B. 1998. State–space modeling of animal movement and mortality with application to salmon. Biometrics, **54**: 1290.

Parma, A.M. 1990. Optimal harvesting of fish populations with non-stationary stock–recruitment relationships. Nat. Resour. Model. **4**: 39–76.

Parma, A.M., and Deriso, R.B. 1990. Experimental harvesting of cyclic stocks in the face of alternative recruitment hypotheses. Can. J. Fish. Aquat. Sci. **47**: 595–610.

Pella, J.J. 1993. Utility of structural time series models and the Kalman filter for predicting the consequences of fishery actions. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse et al. Alaska Sea Grant College Program, Fairbanks, Alaska. pp. 571–593.

Peterman, R.M., Pyper, B.J., Lapointe, M.F., Adkison, M.D., and Walters, C.J. 1998. Patterns of covariation in survival rates of British Columbian and Alaskan sockeye salmon stocks. Can. J. Fish. Aquat. Sci. **55**: 2503–2517.

Reed, W.J., and Simons, C.M. 1996. Analyzing catch–effort data by means of the Kalman filter. Can. J. Fish. Aquat. Sci. **53**: 2157–2166.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Robb, C.A., and Peterman, R.M. 1998. Application of Bayesian decision analysis to the management of a sockeye salmon fishery. Can. J. Fish. Aquat. Sci. **55**: 86–98.

Rosenberg, A.A., and Brault, S. 1993. Choosing a management strategy for stock rebuilding when control is uncertain. Can. Spec. Publ. Fish. Aquat. Sci. No. 120. pp. 243–252.

Schaller, H.A., Petrosky, C.E., and Langness, O.P. 1999. Contrasting patterns of productivity and survival rates for stream-type chinook salmon populations of the Snake and Columbia rivers. Can. J. Fish. Aquat. Sci. **56**: 1031–1045.

Schnute, J.T. 1994. A general framework for developing sequential fisheries models. Can. J. Fish. Aquat. Sci. **51**: 1676–1688.

Sullivan, P.J. 1992. A Kalman filter approach to catch-at-length analysis. Biometrics, **48**: 237–257.

Venrick, E.L., McGowan, J.A., Cayan, D.A., and Hayward, T.L. 1987. Climate and chlorophyll *a* long-term trends in the central North Pacific Ocean. Science (Washington, D.C.), **238**: 70–72.

Walters, C.J. 1986. Adaptive management of renewable resources. Macmillan Publishing Company, New York.

Walters, C.J. 1987. Nonstationarity of production relationships in exploited populations. Can. J. Fish. Aquat. Sci. **44**(Suppl. 2): 156–165.

Walters, C.J. 1990. A partial bias correction factor for stock–recruitment parameters in the presence of autocorrelated environmental effects. Can. J. Fish. Aquat. Sci. **47**: 516–519.

Walters, C.J., and Parma, A.A. 1996. Fixed exploitation rate strategies for coping with effects of climate change. Can. J. Fish. Aquat. Sci. **53**: 148–158.

Appendix. Kalman filter

Our Kalman filter representation of the Ricker model consisted of the following observation and system equations, respectively:

$$\text{(A1)} \quad \log_e(R_t/S_t) = a_t - bS_t + v_t$$

$$\text{(A2)} \quad a_t = a_{t-1} + w_t$$

where the i.i.d. normal error terms v_t and w_t were assumed to be independent. Note that for simplicity of notation in this appendix and in the context of a single age-at-maturity, S_t denotes the spawner abundance that produced the age-m recruits, R, that were observed in year t and the a_t and v_t values apply to that brood class as well. In the context of Bayesian analysis, the Kalman filter provides a recursive procedure for making inferences about the state of a_t, given the observation $\log_e(R_t/S_t)$ (Meinhold and Singpurwalla 1983). Specifically, estimates of the mean ($\hat{a}_t$) and variance (P_t) of the posterior distribution of $\hat{a}_t$ can be computed from the mean ($\hat{a}_{t-1}$) and variance (F_t) of the prior distribution as follows (Meinhold and Singpurwalla 1983):

$$\text{(A3)} \quad \hat{a}_t = \hat{a}_{t-1} + \frac{F_t e_t}{F_t + \hat{\sigma}_v^2}$$

and

$$\text{(A4)} \quad P_t = F_t - \frac{F_t^2}{F_t + \hat{\sigma}_v^2}$$

where

$$\text{(A5)} \quad F_t = P_{t-1} + \hat{\sigma}_w^2$$

and

$$\text{(A6)} \quad e_t = \log_e(R_t/S_t) - (\hat{a}_{t-1} - \hat{b}S_t).$$

The term e_t denotes the error in predicting $\log_e(R_t/S_t)$ based on $\hat{a}_{t-1}$ and $\hat{b}$. To begin the recursive calculations in year $t = 1$, we used initial values of $\hat{a}_{t-1} = 2$ and $P_{t-1} = 1$.

Estimates of b, σ_v, and σ_w were annually updated based on all past data up through the current year $t = N$ by maximizing the following so-called "concentrated" likelihood function (Van Deusen 1989):

$$\text{(A7)} \quad L = -\frac{1}{2}\sum_{t=1}^{t=N} \log_e(F_t + \hat{\sigma}_v^2) + e_t^2/(F_t + \hat{\sigma}_v^2).$$

For computational efficiency in our Monte Carlo trials, we used an equivalent procedure (Visser and Molenaar 1988; Pella 1993) that had two fixed parameters to estimate using maximum likelihood (b and the "signal-to-noise ratio" σ_w^2/σ_v^2), rather than the three parameters in the above steps.

Appendix references

Meinhold, R.J., and Singpurwalla, N.D. 1983. Understanding the Kalman filter. Am. Stat. **37**: 124–127.

Pella, J.J. 1993. Utility of structural time series models and the Kalman filter for predicting the consequences of fishery actions. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse et al. Alaska Sea Grant College Program, Fairbanks, Alaska. pp. 571–593.

Van Deusen, P.C. 1989. A model-based approach to tree ring analysis. Biometrics, **45**: 763–779.

Visser, H., and Molenaar, J. 1988. Kalman filter analysis in dendroclimatology. Biometrics, **44**: 929–940.

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Recovery of fish populations in Lake 223 from experimental acidification

K.H. Mills, S.M. Chalanchuk, and D.J. Allan

Abstract: The fish populations of Lake 223, a lake previously acidified to pH 5.1, were monitored during 13 years of gradual pH recovery to preacidification pH 6.7. During acidification, recruitment ceased for all fish species in this lake and two were extirpated. During pH recovery, successful recruitment resumed for all fish species that remained in the lake. One of the extirpated species, fathead minnow (*Pimephales promelas*), successfully colonized the lake. Lake trout (*Salvelinus namaycush*) abundance decreased during acidification and remained low during pH recovery due to very low recruitment. Growth curves, condition factor, and annual survival of lake trout decreased during acidification and quickly increased to preacidification values during pH recovery. During the early years of pH recovery, white sucker (*Catostomus commersoni*) abundance increased to almost 10 times the number at the start of the experiment but decreased during the final years due to decreased annual survival and recruitment. Pearl dace (*Margariscus margarita*) became abundant during acidification and their abundance decreased during pH recovery as fathead minnow abundance increased. Other fish species that were caught infrequently prior to acidification, brook stickleback (*Culaea inconstans*), lake chub (*Couesius plumbeus*), and finescale dace (*Phoxinus neogaeus*), were caught frequently during pH recovery.

Résumé : Les populations de poissons du lac 223, un lac qui a été acidifié à un pH de 5,1, ont été surveillées pendant les 13 ans qu'a duré la remontée du pH jusqu'à sa valeur antérieure de 6,7. Durant l'acidification, le recrutement s'est arrêté pour toutes les espèces de poissons du lac, et deux espèces ont disparu. Pendant la remontée du pH, le recrutement a recommencé avec succès pour toutes les espèces de poissons qui restaient dans le lac. Une des espèces disparues, la tête-de-boule (*Pimephales promelas*), a été réintroduite avec succès. L'abondance de touladi (*Salvelinus namaycush*) a diminué pendant l'acidification et est restée basse durant la remontée du pH à cause d'un très faible taux de recrutement. Les courbes de croissance, le cœfficient de condition et le taux de survie annuel du touladi ont baissé durant l'acidification, mais sont rapidement revenus à leurs valeurs précédentes pendant la remontée du pH. Au cours des premières années de remontée du pH, l'abondance du meunier noir a augmenté de presque dix fois par rapport à sa valeur initiale, mais a diminué pendant les dernières années à cause de la baisse du taux annuel de survie et de recrutement. L'abondance de mulet perlé (*Margariscus margarita*) a augmenté durant l'acidification, mais a diminué pendant la remontée du pH, parallèlement à l'augmentation de l'abondance de la tête-de-boule. D'autres espèces de poisson qui étaient pêchées sporadiquement avant l'acidification - l'épinoche à cinq épines (*Culaea inconstans*), le méné de lac (*Couesius plumbeus*) et le ventre citron (*Phoxinus neogaeus*) - ont été capturées fréquemment pendant la remontée du pH.

[Traduit par la Rédaction]

Introduction

The adverse effects of lake acidification on fish populations in Europe and North America are well known (Muniz and Leivestad 1980; Baker et al. 1990). We know also that significant reductions in acidic deposition will be followed by pH recovery of many lakes (Allott et al. 1992; Keller et al. 1992; Havas et al. 1995), but the rate and extent of biological recovery that follows this pH recovery are not well known or thought to take decades (Keller and Yan 1998). Lake 223, the Experimental Lakes Area (ELA), northwestern Ontario (Cleugh and Hauser 1971), was experimentally acidified from 1976 to 1983 (Schindler et al. 1985). Starting in 1984, we allowed the pH of Lake 223 to gradually recover to preacidification pH. The purpose of this paper is to describe changes in the Lake 223 fish populations during this pH recovery from 1984 to 1996.

Mills et al. (1987) described changes in the fish populations of Lake 223 during 8 years of acidification. We tested hypotheses during pH recovery that were linked to changes that occurred during acidification. Although Lake 223 was intentionally acidified for only 8 years, 1976–1983, changes in fish populations were similar to those documented in lakes acidified for decades by atmospheric precipitation (Mills and Schindler 1986; Mills et al. 1987; Baker et al. 1990). We believe that the changes that occurred in Lake 223 fish populations during pH recovery may be representative of the changes that could occur as other moderately acidified lakes recover.

We identified pH thresholds where recruitment failures started for five Lake 223 fish species during acidification: 5.9 for fathead minnow (*Pimephales promelas*), 5.6–5.9 for

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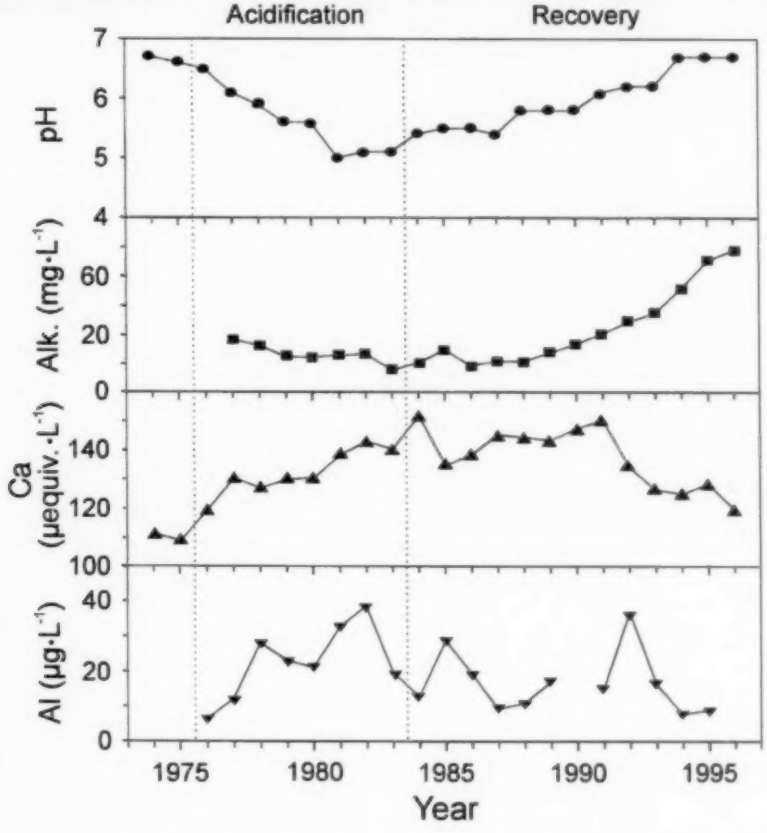
Fig. 1. Open-water annual means for pH, Gran alkalinity, calcium, and total aluminum at 1 m depth in Lake 223.



slimy sculpin (*Cottus cognatus*), 5.6 for lake trout (*Salvelinus namaycush*), 5.1 for pearl dace (*Margariscus margarita*), and 5.0 for white sucker (*Catostomus commersoni*) (Mills et al. 1987). Slimy sculpin and fathead minnow became extinct during acidification, but adults of the other three species were present at the start of pH recovery. We hypothesized that recruitment would resume for each of the remaining fish species when the epilimnetic pH increased above the pH thresholds where recruitment failures started during acidification. We hypothesized that abundance of each species would increase rapidly after recruitment resumed.

Lake trout condition, growth curves, and annual survival decreased during the final years of acidification. We believed that these changes were caused by decreased abundance of lake trout prey species: *Mysis relicta*, fathead minnow, pearl dace, slimy sculpin, and juvenile white sucker (Mills et al. 1987). We hypothesized that condition, growth, and annual survival of lake trout would quickly increase during pH recovery as abundance of prey species increased.

Increased recruitment during the early years of acidification caused the abundance of white sucker to more than double, even though recruitment failures occurred during the final 3 years of acidification from 1981 to 1983 (Mills et al. 1987). Condition of white sucker increased and then decreased so that condition in 1983 was slightly less than at the start of the experiment. We hypothesized that white sucker abundance would decrease during pH recovery and that this decrease would be due to increased predation by a more abundant lake trout population. We also hypothesized that white sucker growth curves would decrease during pH recovery to values similar to those prior to acidification, while white sucker condition would change little during pH recovery.

Two fish species, fathead minnow and slimy sculpin, became extinct during acidification. Although each species occurs in the lake directly upstream of Lake 223, there is frequently no flow between this upstream lake and Lake 223. We hypothesized that fathead minnow and slimy sculpin would not colonize Lake 223 during pH recovery and the Lake 223 fish community would be composed of the species that were present in 1983 at the end of the acidification phase of the experiment: lake trout, white sucker, and pearl dace.

Materials and methods

Study lakes

Lake 223 is a small lake (27.3 ha, 14.4 m z_{max}) in the ELA (Cleugh and Hauser 1971). It was gradually acidified with electrolyte-grade sulfuric acid from 1976 (epilimnetic pH 6.5) to 1981 (pH 5.0) (Fig. 1). Lake acidity was maintained at pH 5.0–5.1 in

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1982 and 1983. We allowed the pH of Lake 223 to gradually increase in three stages, pH 5.4 (1984–1987), pH 5.8 (1988–1990), and pH 6.1 (1991–1993), before reaching preacidification pH 6.7 (1994–1996). No acid-neutralizing chemicals were added to increase lake pH during recovery. A combination of runoff during spring melt and internal alkalinity generation (Cook et al. 1986) increased pH. Internal alkalinity generation was so efficient that sulfuric acid was added to ensure that lake pH did not increase above the target values from 1984 to 1993. No acid was added to the lake from 1994 to 1996. Lake pH immediately increased to preacidification pH 6.7 in 1994 and remained at this value through 1996.

Although only sulfuric acid was added to Lake 223, other changes occurred in the water chemistry of the lake throughout acidification and pH recovery (Fig. 1) (ELA staff, unpublished data). Aluminum concentrations increased during acidification and decreased during pH recovery with the exception of 1992. Aluminum concentrations and toxicity generally increase as lakes and rivers acidify, but values in Lake 223 were less than toxic levels reported in other bioassay and synoptic survey studies (Baker et al. 1990). Aluminum concentrations remained low because acid was added directly to the lake rather than to the watershed surrounding the lake. The calcium content of water is an ameliorating factor for acid and aluminum toxicity for aquatic organisms. Unlike atmospherically acidified lakes where calcium concentrations usually decrease during acidification, calcium concentrations increased as Lake 223 was acidified. They decreased during the final years of pH recovery, but the overall change was unlikely to have had an impact on resistance of Lake 223 organisms to toxicity because the concentrations were relatively high throughout the experiment when compared with atmospherically acidified lakes (Baker et al. 1990).

The fish populations in natural, unacidified lakes 224 (25.4 ha, 16.7 m z_{max}) and 468 (300.8 ha, >25 m z_{max}) were monitored to compare with Lake 223 results. Both were pristine lakes with epilimnetic pH from 6.8 to 7.0 and had fish communities similar to Lake 223 (Beamish et al. 1976; Mills et al. 1987; ELA staff, unpublished data). We utilized lake trout, fathead minnow, pearl dace, and slimy sculpin data from Lake 224 and white sucker data from Lake 468 to compare with Lake 223 data.

Fish capture and sampling

Lake trout in lakes 223 and 224 and white sucker in Lake 223 were captured from 1984 to 1998 using the same techniques (trap nets, gill nets, and angling) that were used and described by Mills et al. (1987) for the acidification phase of the experiment. Individuals were weighed, measured (fork length), marked (external numbered tags, fin-ray scarring), and released following the methods described in Mills et al. (1987). Commencing in 1988, lake trout were also marked with Visible Implant (V.I.)® tags (Haw et al. 1990). We aged lake trout and white sucker by pectoral or pelvic fin rays using the methods and age validation technique described in Mills and Beamish (1980) and Chalanchuk (1984). Capture and handling mortality was <4% for lake trout and white sucker from 1975 to 1996. White sucker were captured in Lake 468 in 1973, 1984–1986, and 1988–1998 using overnight sets of multipanel gill nets each year as described in Mills et al. (1987).

Other fish species in lakes 223 and 224 were captured in trap nets fished in September and October of each year. Each fish was anesthetized and measured (fork length for all species). Average catch-per-unit-effort was used to compare abundance of these species among years for each lake. We defined a unit of effort as one overnight set of a trap net. The average effort used each fall to capture these species from 1984 to 1996 in each lake was approximately 100 net-days·year^{-1}. Although many fathead minnow were caught in Lake 223 in 1977, we did not record the number caught during fall sampling. Catches were similar to those the preceding year, 1976, and we used this number as an estimate of catch-per-unit-effort for 1977. Similarly, we caught pearl dace in Lake 223 during 1976 and 1977 but did not count or sample this species during these years. We captured few individuals in trap nets and we estimated that catch-per-unit-effort was lower in 1976 and 1977 than any other year of study in Lake 223.

Recruitment

Because we determined the age of each lake trout and white sucker captured throughout the entire experiment, we used these data as a basis to calculate recruitment of year-classes for each species. We defined recruitment of a year-class as the total number of fish caught from that year-class during all years of sampling. Because fish were marked at first capture in lakes 223 and 224, we could easily avoid double counting individual fish in the total for a particular year-class. The yearly fishing effort, two overnight sets of gill nets, in Lake 468 was approximately an order of magnitude less than the yearly effort in lakes 223 and 224 using trap nets, gill nets, and angling. Therefore, no direct comparisons of the magnitude of white sucker year-classes between lakes are possible. The yearly fishing effort for lake trout in lakes 223 and 224 was almost identical, so direct comparisons between lakes were possible.

We calculated annual recruitment of pearl dace and fathead minnow as the catch-per-unit-effort for each species captured during fall trap netting of each year. Based on length–frequency distributions collected each spring and fall, age-0 (young-of-the-year) pearl dace were <70 mm fork length during fall sampling in Lake 223. Age-0 fathead minnow were <40 mm at this time, and individuals <30 mm were not sampled effectively by our trap nets. Therefore, we used catch-per-unit-effort of age-1 fathead minnow (40–60 mm) and age 0 pearl dace as measures of annual recruitment for these species. Tallman et al. (1984) and Chalanchuk et al. (1989, 1991) found the same correspondences of ages to length groups using length–frequency distributions for fathead minnow and pearl dace populations in other ELA lakes. Although we did not count or measure pearl dace in 1976 or 1977, the total number caught each year was lower than in any subsequent year, so we estimated that recruitment in 1976 and 1977 was also lower than in subsequent years. Similarly, because we did not measure any fathead minnow in 1977, we could not quantify recruitment of the 1976 year-class but estimated this to be similar to that of the 1977 year-class.

Condition, growth, abundance, and survival of lake trout and white sucker

Annual lake trout and white sucker condition, growth curves, and survival estimates were calculated in the same manner as reported for the acidification phase of the experiment (Mills et al. 1987). Average condition factors (k) and their 95% confidence intervals were calculated as $k = 10^5 w/l^3$ where w is weight (grams) and l is fork length (millimetres) (Bagenal and Tesch 1978). Growth curves were constructed from mean fork length data for individual age groups. Abundance and annual survival estimates were calculated using the Jolly–Seber "full" model (Jolly 1965; Seber 1982) incorporating the correction for small sample sizes as recommended by Arnason and Baniuk (1978). Individuals were fully recruited into the mark–recapture pool at age 1+ (fall sampling periods). We used mark–recapture data from successive fall sampling periods for lake trout estimates and from successive spring sampling periods for white sucker estimates. Because a Jolly–Seber survival estimate is the survival of fishes from release in one sampling period to the start of the subsequent sampling period, we describe an individual estimate by a dual-year designation (e.g., 1983/84).

We included growth curves, condition, abundance, and annual survival estimates from the acidification phase of the experiment (Mills et al. 1987) in some figures to facilitate comparisons with

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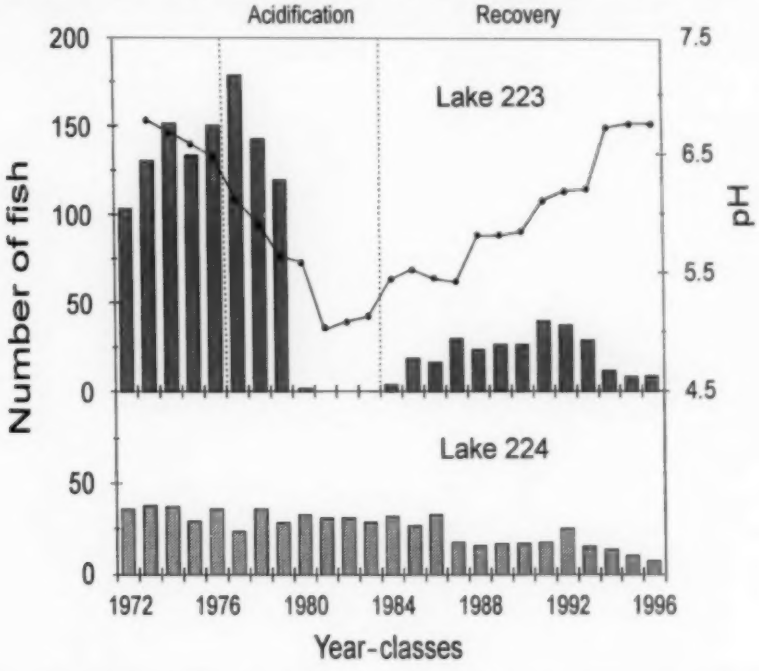
Fig. 2. Catches of the 1972–1996 year-classes of lake trout in Lake 223 and unmanipulated Lake 224.



results during pH recovery. Some of the estimates for abundance and annual survival of lake trout and white sucker for the acidification phase of the experiment are slightly different from those reported in Mills et al. (1987). These changes are due to additional recaptures of individuals released during the acidification phase of the experiment. In addition, we converted the 2-year annual survival estimate for Lake 224 lake trout from 1976 to 1978 (Mills et al. 1987) to single-year estimates, 1976/77 and 1977/78, using Seber's (1982) delta method described in Mills and Chalanchuk (1987). We analyzed some previously unpublished fish survey data from 1973 for lake trout in Lake 223 and white sucker in Lake 468 (Beamish et al. 1976) to obtain condition estimates for 1973.

Abundance of pearl dace, fathead minnow, and other fishes

We calculated pearl dace and fathead minnow abundance as average catch-per-unit-effort for all individuals caught during fall sampling periods. We monitored the species composition of trap net catches in lakes 223 and 224 during spring and fall sampling of each year. In particular, we examined Lake 223 catches for the presence of fathead minnow and slimy sculpin because each was present in upstream Lake 224 and individuals could potentially immigrate to Lake 223 in the stream connecting the two lakes. When fathead minnow first appeared in Lake 223 catches, we placed commercial minnow traps in the stream connecting lakes 223 and 224 in early spring 1985 that confirmed that fathead minnow were present in this stream during the high flows of spring snowmelt.

Results

Recruitment

Successful recruitment of lake trout in Lake 223 (Fig. 2) started during pH recovery in 1984 (pH 5.4), but year-classes averaged only 20 individuals per year, while the average was 137 before recruitment failures started during acidification. The 1984 year-class consisted of only three individuals and was the smallest year-class observed during pH recovery. The number of lake trout in each Lake 224 year-class was relatively consistent from 1972 to 1996, although recruitment during the most recent 10 years was slightly lower than during earlier years.

Recruitment of white sucker year-classes in Lake 223 (Fig. 3) resumed in 1984 (pH 5.4), but the abundance of year-classes varied by more than two orders of magnitude during subsequent years. The variability was highest during the initial years of pH recovery due to the abundant 1984 and 1986 year-classes. Recruitment decreased during the final 2 years of this study to the lowest value recorded during the entire study in 1996. Recruitment of white sucker year-classes in Lake 468 was also variable, but differences were less than one order of magnitude between abundant and weak year-classes. Excluding the 3 years of recruitment failures in Lake 223 at low pH, there was some correspondence of strong year-classes between lakes 223 and 468. The 1978, 1984, and 1986 year-classes were very abundant in each lake.

Recruitment resumed for pearl dace in 1984 (pH 5.4) (Fig. 4) and for fathead minnow in 1988 (pH 5.8). Recruitment of pearl dace during the final 2 years of pH recovery, 1995 and 1996, was less than recruitment during other years of pH recovery. Although adult fathead minnow were caught infrequently starting in 1986 (pH 5.4), young-of the-year were not captured until 1988 (pH 5.8), when six individuals were caught. Progressively more fathead minnow recruits were captured as pH increased from pH 5.8 (1988–1990) to 6.7 (1994–1996). Recruitment of pearl dace and fathead minnow occurred each year, 1984–1996, in Lake 224, but catch-per-unit-effort averaged <3 individuals for each year

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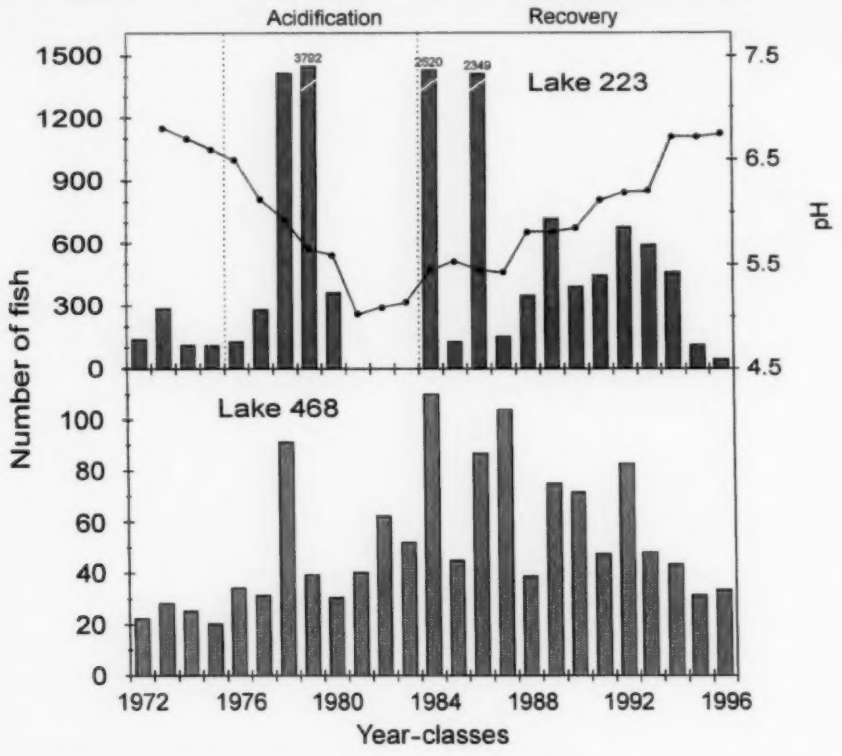
Fig. 3. Catches of the 1972–1996 year-classes of white sucker in Lake 223 and unmanipulated Lake 468.



of study. Although catches were much lower in Lake 224 than in Lake 223, successful recruitment occurred each year in Lake 224.

Lake trout condition, growth curves, and annual survival

Average condition of lake trout in Lake 223 increased in 1984, the first year of pH recovery, and continued at values similar to those prior to acidification in 1973 and 1975 (Fig. 5) for the remainder of this study. Lake trout condition in unacidified Lake 224 increased from 1983 to a maximum in 1985 and then gradually decreased throughout the remainder of the study to reach levels similar to that in 1978 and 1979.

Growth curves of Lake 223 lake trout gradually increased from 1984 to 1996 (representative curves are shown in Fig. 6). In general, mean lengths of young, immature Lake 223 lake trout (age ≤ 5) were greater during pH recovery than those of fish of similar ages in Lake 223 either prior to or during the acidification phase of the experiment. Growth curves of Lake 224 lake trout were almost identical from 1983 to 1996.

Annual survival of lake trout in Lake 223 increased during the initial years of pH recovery (Fig. 7) and then remained above 0.8 (80%) per year for the rest of the study. The annual survival of lake trout from the fall of 1983 to the fall of 1984 (1983/84), 0.5 ± 0.07, is much lower than that reported by Mills et al. (1987), 0.67 ± 0.1, for the same time interval. The estimates for some years of pH recovery were >1.0 (>100% annual survival). These estimates indicate extremely high survival for these years rather than problems with the model or biases in the data. Annual survival of lake trout in Lake 224 was ≥ 0.08 (80%) per year from 1983/84 to 1995/96 and there were no significant differences between estimates (Z tests, $P > 0.5$).

Lake trout abundance

Abundance of Lake 223 lake trout significantly decreased from 1984 to 1986 (Z tests, $P < 0.05$), stabilized from 1986 to 1993 at about 200 individuals, and then gradually increased starting in 1993 (Fig. 7). The largest increase occurred in 1996 when abundance increased to more than 325 individuals. Lake trout abundance in reference Lake 224 was very stable, approximately 300 individuals, from 1983 to 1996. There were no significant differences among abundance estimates (Z tests, $P > 0.1$) of lake trout in Lake 224.

White sucker abundance, annual survival, growth curves, and condition

Abundance of Lake 223 white sucker decreased from 1984 to 1985, increased in 1986, peaked in 1989, and decreased during the latter years of pH recovery (Fig. 8). Although the 1984 year-class was very abundant, these individuals were not recruited into the mark–recapture pool until fall 1985 at age 1+ and were responsible for the large increase in abundance in spring 1986. The decrease in abundance was particularly notable from 1995 to 1996 (75%) so that the 1996 value was not significantly different (Z test, $P > 0.58$) from the abundance at the start of acidification in 1976.

Annual survival of white sucker was generally higher during the initial years of pH recovery than during the latter

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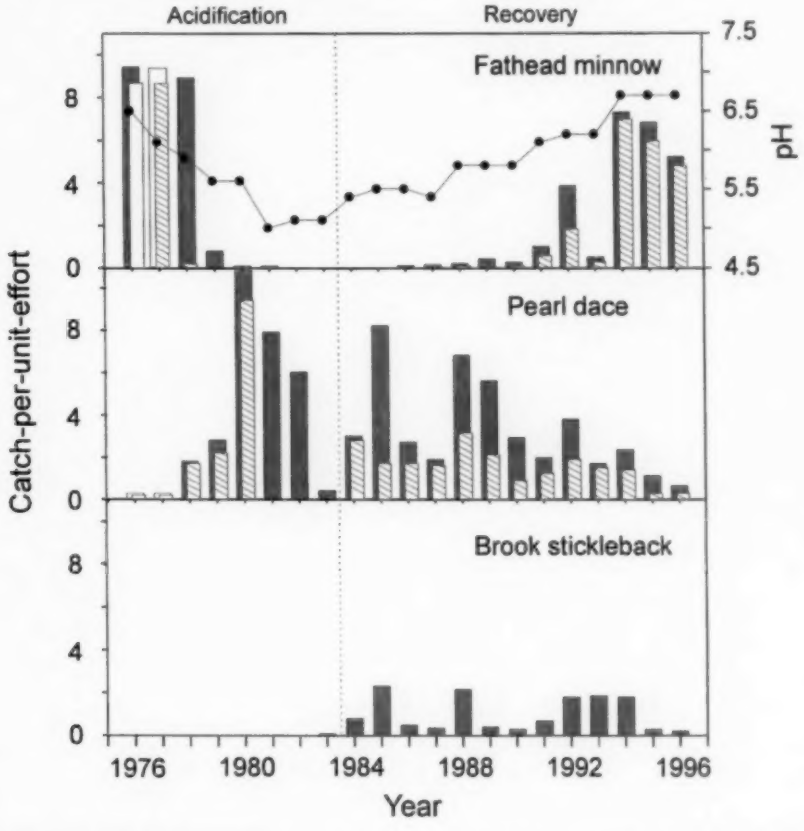
Fig. 4. Catch-per-unit-effort of fathead minnow, pearl dace, and brook stickleback for all age-classes (stippled bars). Age-0 fathead minnow and pearl dace are indicated by hatched bars in Lake 223 from 1976 to 1996. Extrapolated estimates are indicated by open bars. See text for details.



years (Fig. 8). With the exception of 1987/88, annual survival averaged almost 80% each year from 1984/85 to 1990/91. This was almost 20% higher than the average, 67%, for all years of the acidification phase of the experiment. Annual survival declined in 1991/92 and further declined in 1995/96 when the lowest annual value, 45% (0.45 ± 0.07), occurred. This value was significantly less (*Z* test, $P < 0.05$) than the previous lowest value, 58% (0.58 ± 0.06), recorded for 1981/82.

Growth curves of Lake 223 white sucker decreased throughout all but the final 2 years of pH recovery (representative years are shown in Fig. 9). At the end of the acidification phase of the experiment in 1983, white sucker mean lengths at age were greater than at the start of the experiment in 1975. Mean lengths at age progressively decreased during the recovery phase of the experiment from 1984 to 1994 and then stabilized until 1996. Mean lengths at age were lower in 1994 than in any previous year of this study (ANOVA, $P < 0.02$). Growth curves of Lake 468 white sucker were almost identical among years of this study (representative years are shown in Fig. 9). Mean lengths at age for Lake 468 white sucker were not significantly different among years (ANOVA, $P > 0.53$).

Condition of Lake 223 white sucker was lower during most years of pH recovery than during the years of acidification or prior to acidification (Fig. 5). Exceptions occurred in 1984, 1985, and 1996. The 1984, 1985, and 1996 values were not significantly different (*t* tests, $P > 0.9$) from that at the start of the experiment in 1976. Almost all white sucker were emaciated during the years of lowest condition: 1987, 1990, and 1991. Condition of Lake 468 white sucker varied much less than that of Lake 223 white sucker (Fig. 5). There was more variation in estimates from 1988 to 1994 than during the earlier years of study for Lake 468 fish, but all values were not significantly different ($P > 0.3$) from the value for 1973.

Fathead minnow, pearl dace, slimy sculpin, and other fish species

Fathead minnow, a species that was extirpated from Lake 223 during the acidification phase of the experiment, was caught in Lake 223 during pH recovery (Fig. 4). One individual was captured in 1986. Catches gradually increased as pH increased until 1994 (pH 6.7) when the largest increase occurred. Fathead minnow recruitment started during pH recovery in 1988 (pH 5.8), and incremental increases occurred as pH increased to 6.1 and 6.7.

Pearl dace abundance (Fig. 4) increased during pH recovery from the low value of the final year of acidification, 1983. Although mean catch-per-unit-effort was quite vari-

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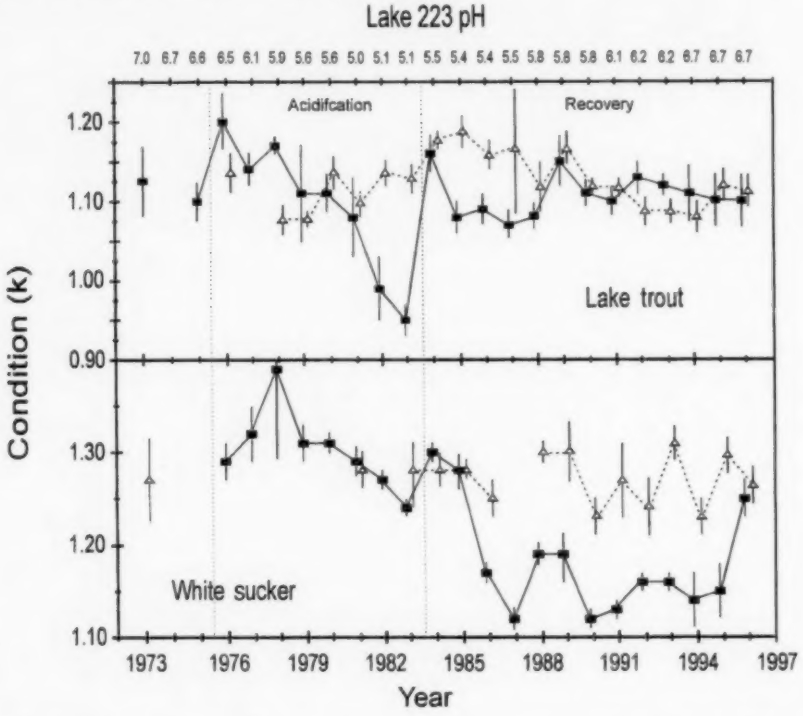
Fig. 5. Average condition of lake trout and white sucker from Lake 223 (squares) and unmanipulated lakes 224 and 468 (triangles). Means and 95% confidence intervals are presented. Sample sizes for lake trout were 3934 (Lake 223) and 2836 (Lake 224). Sample sizes for white sucker were 28 140 (Lake 223) and 1211 (Lake 468).



able, there was an overall decline throughout pH recovery. The two lowest values occurred in the final 2 years of pH recovery.

Brook stickleback (*Culaea inconstans*), a species first caught during the final year of acidification in Lake 223, was frequently caught in trap nets during all years (Fig. 4). Two other fish species, lake chub (*Couesius plumbeus*) and finescale dace (*Phoxinus neogaeus*), were caught frequently during pH recovery, although catch-per-unit-effort was generally <0.25·net-day^{-1}. We identified lake chub in our trap net catches starting in 1986 and caught 87 individuals during this year in Lake 223. We caught this species each year throughout the remainder of the recovery period but averaged fewer than 20 individuals as the yearly total catch. Only four finescale dace were caught in Lake 223 during the acidification phase of the experiment: one was caught in 1980 and three were caught in 1983. We captured a few individuals during each year of pH recovery, but the largest yearly catch was 22 in 1992 and the smallest catch was one individual in 1987 and 1996.

Discussion

Recruitment

Our hypothesis that recruitment of Lake 223 fishes would resume during pH recovery was confirmed. Recruitment for each species resumed at approximately the same pH where recruitment ceased during the acidification phase of the experiment.

Lake trout recruitment ceased during the acidification phase of the experiment when lake pH was 5.6 in 1980 (Mills et al. 1987) and resumed when lake pH increased to 5.4 in 1984 (Fig. 2). Although the open-water pH means for 1979 and 1980 were identical, 5.6, the pH values during the fall spawning period and winter were lower in 1980, 5.3–5.6, than in 1979, 5.6–5.8. Recruitment failures of lake trout can be attributed to mortality of sac fry during winter (Gunn and Keller 1984), so it is not surprising that there was an almost complete year-class failure of lake trout in Lake 223 in 1980 but not in 1979. The pH threshold for resumption of lake trout recruitment in Lake 223 is similar to the pH value, 5.5, that Gunn and Keller (1990) and Keller et al. (1992) reported for lake trout recruitment in Whitepine Lake, Ontario, while this lake was undergoing natural pH recovery.

The 1984 year-class was the smallest of all lake trout year-classes during pH recovery (Fig. 2), although the average ice-free lake pH during this year was similar to that of the 3 subsequent years. There are at least two possible explanations for the small 1984 year-class. First, the 1984 year-class was the result of eggs deposited during fall 1983 and incubated during winter when lake pH was 5.1–5.2. Sac fry mortality was likely higher during the 1983/84 winter than during subsequent winters when lake pH was ≥5.4 (ELA staff, unpublished data). Second, cannibalism might be an important factor suppressing lake trout recruitment this first year of pH recovery. Few prey items were available to

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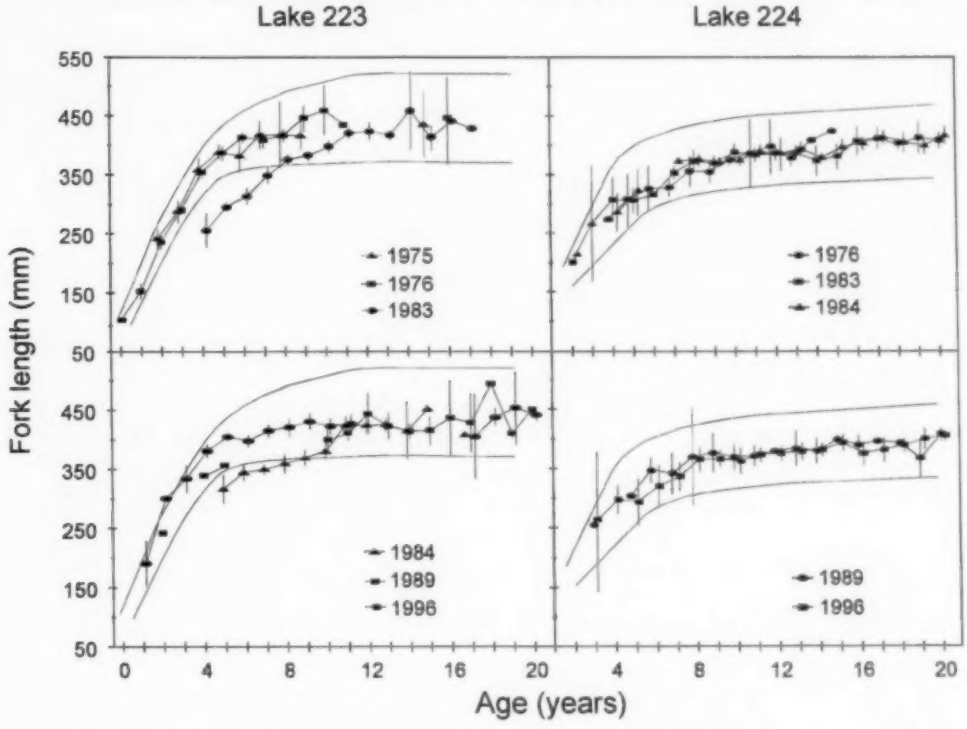
Fig. 6. Representative growth curves for lake trout in Lake 223 and unmanipulated Lake 224. Mean lengths and 95% confidence intervals are plotted. Curves for preacidification (1975, pH 6.7) and the first (1976, pH 6.5) and final (1983, pH 5.8) years of the acidification phase of the experiment in Lake 223 are taken from Mills et al. (1987). Curves for the first (1984, pH 5.4), sixth (1989, pH 5.8), and 13th (1996, pH 6.7) years of pH recovery are presented. Sample sizes for Lake 223 varied from 55 to 212 and for Lake 224 from 68 to 183 for individual years. To facilitate comparisons between years, the upper and lower limits of lake trout growth for years prior to pH decline in Lake 223, 1975–1976 (from Mills et al. 1987), and the earliest years of Lake 224 data, 1976 and 1978, are also presented.



adult lake trout in 1982 and 1983 and lake trout were emaciated (Schindler et al. 1985; Mills et al. 1987). Although many age-0 white sucker and pearl dace were present during the first year of pH recovery in 1984 (Figs. 3 and 4), these appeared later in the year than age-0 lake trout. Cannibalism of adult lake trout on juveniles occurred during 1982 and 1983 (Mills et al. 1987) and could have occurred early in 1984. Cannibalism is an important factor regulating arctic populations of lake trout (Johnson 1976). Because many small white sucker, pearl dace, fathead minnow, and brook stickleback were present in Lake 223 after 1984, cannibalism was likely less important for affecting subsequent lake trout year-classes.

White sucker recruitment ceased during the acidification phase of the experiment when average lake pH decreased from 5.6 (1980) to 5.1 (1981) and resumed when lake pH increased from 5.1 (1983) to 5.4 (1984) (Fig. 3). The magnitude and variability of year-classes during pH recovery surprised us. There may be a combination of factors responsible for high recruitment during many of the years. In general, white sucker year-classes were larger in Lake 468 from 1984 to 1996 (mean = 62) than in previous years (mean = 40), so climatic factors may be partially responsible for the larger year-classes in Lake 223 during pH recovery. Other factors, such as reduced lake trout predation due to low abundance of adult lake trout during pH recovery (Fig. 7), may be important. During the acidification phase of the experiment, white sucker recruitment was very high, and Mills et al. (1987) hypothesized that improved nursery habitat, caused by the appearance of extensive mats of *Mougeotia*, was partially responsible for this change. During pH recovery, the *Mougeotia* algal mats receded and this may be related to the decrease in white sucker recruitment during the final 2 years of this study.

Pearl dace recruitment ceased during the acidification phase of the experiment in the second year that lake pH was held at pH 5.0–5.1 (1982) and resumed in the first year that lake pH increased to 5.4 (1984) (Fig. 4). Successful recruitment occurred the first year the average annual pH was 5.0 (1981), but no recruitment occurred the two subsequent years at similar pH values. This may seem a contradiction, but we believe that the explanation for the difference in pH thresholds is that lake pH at the time of pearl dace spawning in 1981 was higher than the annual average pH for that year. Pearl dace spawn at ice-off at the ELA. Acid additions usually started each year the second or third week after ice-off in Lake 223. Although the average yearly pH of Lake 223 was 5.0 in 1981, the lake pH at ice-off before acid additions was 5.8. This pH was above the threshold for recruitment failure for pearl dace, pH 5.4, based on synoptic surveys,

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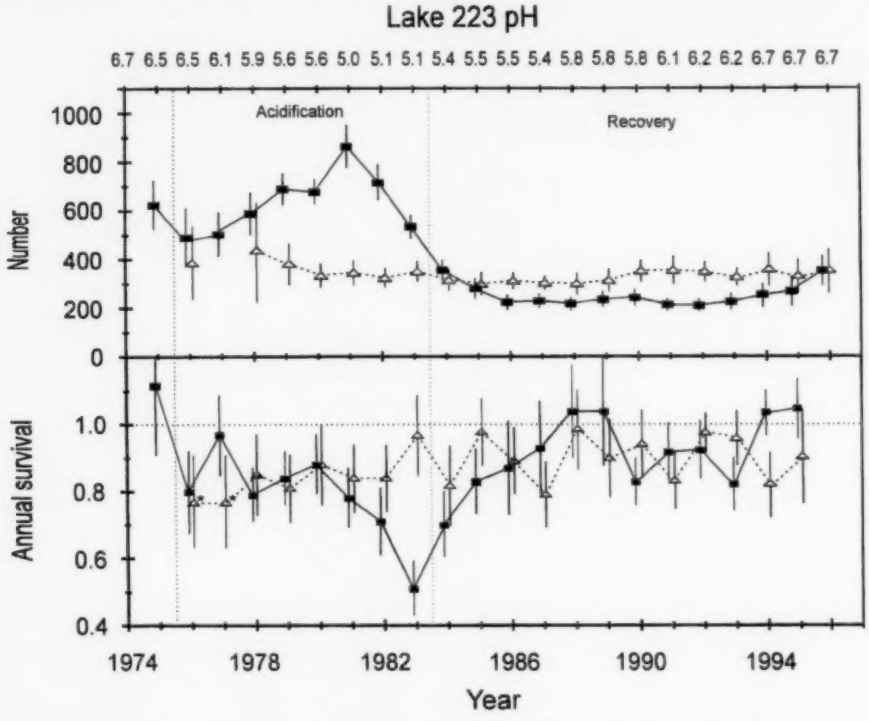
Fig. 7. Abundance and annual survival of lake trout in lakes 223 (squares) and 224 (triangles). Jolly–Seber full model (Jolly 1965; Seber 1982) estimates and their 95% confidence intervals are plotted except for the initial abundance estimates for each population that were calculated using the death-only model. Abundance estimates are formed for fall sampling periods of each year. Annual survival estimates are estimates from the fall sampling period of a year to the fall sampling period of the following year.



(Mills et al. 1987). Therefore, it is not surprising that pearl dace recruitment occurred in 1981 although the average annual pH for that year was 5.0. The pH at ice-off in 1982 and 1983, pH 5.1, was below the threshold and no recruitment occurred.

Lake trout condition, growth, and annual survival

Our hypothesis that lake trout condition, growth curves, and annual survival would increase rapidly during pH recovery as abundance of prey species increased was partially confirmed. Lake trout condition (Fig. 5) and annual survival (Fig. 7) increased rapidly to preacidification values during the initial years of pH recovery, but lake trout growth curves (Fig. 6) increased slowly. During the acidification phase of the experiment, growth curves and condition decreased concomitantly from 1978 to 1993 (Mills et al. 1987). Mills et al. (1987) explained the decreases by the extinction of fathead minnow (Fig. 4), *Mysis relicta*, and slimy sculpin and by abundance decreases of small white sucker (Fig. 3) and pearl dace (Fig. 4). We expected that condition and growth curves would quickly increase together during pH recovery when prey species became abundant. Two of the primary prey species, white sucker and pearl dace, resumed recruitment in 1984 and became very abundant. In addition, brook stickleback abundance increased during pH recovery (Fig. 4). This species is a preferred lake trout prey species (Scott and Crossman 1973). In addition, we expected that the low abundance of lake trout might accelerate growth curve increases due to density dependence, as reported for Lake Michigan lake trout (Ferreri and Taylor 1996). Although mean lengths at age of adult lake trout did gradually increase during pH recovery, we had thought that conditions were ideal for a more rapid increase in growth curves.

Lake trout abundance

Our hypothesis that lake trout abundance would rapidly increase during pH recovery was rejected. Although recruitment resumed immediately after lake pH increased above the threshold for reproductive failure in 1984, recruitment remained very low. Lake trout abundance decreased during the early years of pH recovery (Fig. 7) and remained approximately 50% lower throughout the pH recovery period than that prior to acidification. There is no doubt that the cause of the low abundance was low recruitment because annual survival of individuals was high (Fig. 8). We were very surprised that recruitment remained low throughout the recovery period because increased recruitment is frequently a response of many fishes when abundance of adults is reduced by exploitation (Healey 1980; Mills et al. 1995; Ferreri and Taylor 1996). Healey (1978) suggested that lake trout may show recruitment compensation to increased exploitation, but he cautioned that there was little direct evidence to confirm this.

White sucker abundance, annual survival, growth curves, and condition

We hypothesized that the abundance of white sucker

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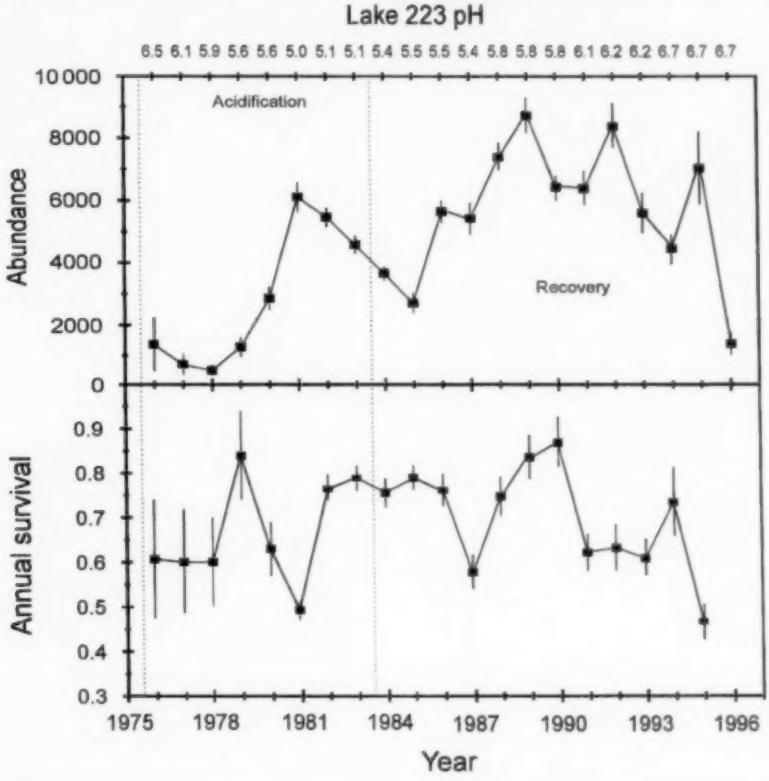
Fig. 8. Abundance and annual survival of white sucker in Lake 223. Jolly–Seber full model estimates (Jolly 1965; Seber 1982) and their 95% confidence intervals are plotted. Abundance estimates are formed for spring sampling periods of each year. Annual survival estimates are estimates from the spring sampling period of a year to the spring sampling period of the following year.



would decrease during pH recovery and that this decrease would be caused by increased lake trout predation as lake trout abundance increased. Although abundance of white sucker did finally decrease in 1996 to a number not significantly different from that at the start of the acidification phase of the experiment in 1976, this decrease occurred abruptly due to low annual survival (Fig. 8) coupled with low recruitment in 1995 and 1996 (Fig. 3). It is unlikely that lake trout predation caused this change because lake trout abundance remained low throughout pH recovery.

We were not surprised that annual survival of Lake 223 white sucker finally decreased. Other characteristics of the population, greatly decreased growth curves (Fig. 9) and low condition (Fig. 5), mirrored these population parameters when lake trout annual survival decreased during the acidification phase of the experiment (Mills et al. 1987). Most white sucker were extremely emaciated in 1987 and 1990–1995.

Our hypothesis of decreased white sucker growth curves and condition during pH recovery was confirmed, although we were very surprised that growth decreased to such low values. The abundance of zoobenthos is a good predictor of white sucker growth curves (Chen and Harvey 1995). Dipterans are a primary food of white sucker at the ELA, and the biomass of dipterans emerging from Lake 223 decreased during pH recovery (I.J. Davies, ELA staff, unpublished data). Overharvesting their food supply is the likely reason that white sucker growth curves and condition decreased to extremely low values during pH recovery.

Fish colonization

Contrary to our hypothesis, fathead minnow became reestablished in Lake 223 during pH recovery. We believe that this species emigrated from Lake 224 to downstream Lake 223. Fathead minnow are usually thought to live a maximum of 3–5 years (Scott and Crossman 1973; Tallman et al. 1984). The pH of Lake 223 was below the threshold for reproductive failure of this species, 5.8–6.0 (Mills et al. 1987; McCormick et al. 1989; Baker et al. 1990), for 9 years. During this 9-year period, we fished small-mesh trap nets each spring and fall for over 300 net-nights each year. The last fathead minnow individuals that we caught during the acidification phase of the experiment in 1980 and 1981 were all extremely large, old individuals. We believe that it is extremely unlikely that a few individuals survived the entire 9 years that lake pH was below the threshold for successful reproduction of this species to replenish the population when lake pH increased to pH 5.8 during pH recovery. Based on our fathead minnow catches in the stream connecting lakes 223 and 224, we believe that Lake 224 was the seed population for the colonization of Lake 223.

We do not know whether brook stickleback and finescale dace colonized Lake 223 from Lake 224 during the latter years of acidification or whether these species were present

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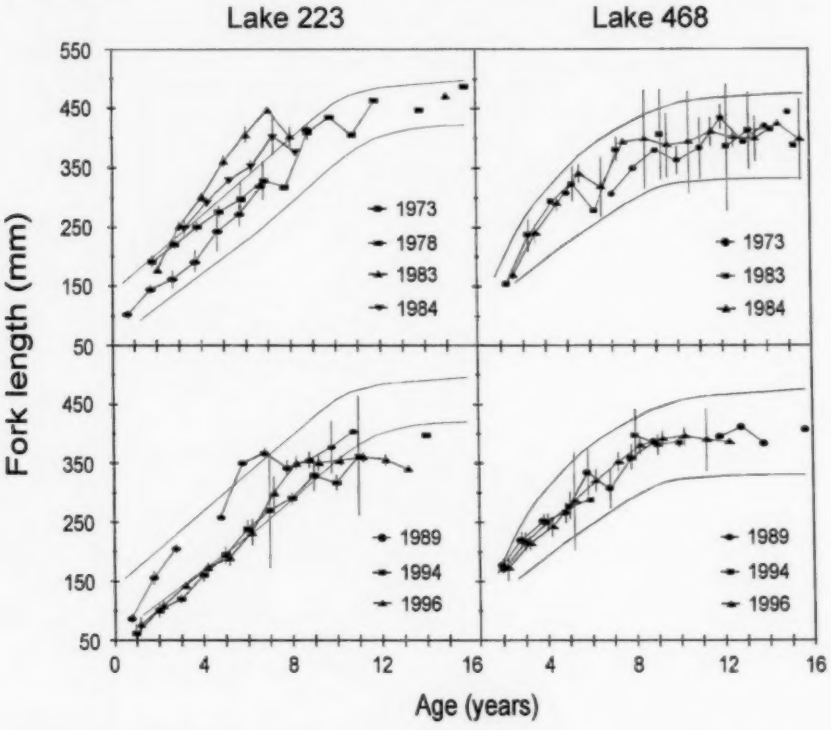
Fig. 9. Representative growth curves for white sucker in Lake 223 and unmanipulated Lake 468. Mean lengths and 95% confidence intervals are plotted. Curves for preacidification (1973, pH 7.0) and the third (1978, pH 5.9) and final (1983, pH 5.1) years of the acidification phase of the experiment in Lake 223 are taken from Mills et al. (1987). Curves for the first (1984, pH 5.4), sixth (1989, pH 5.8), 11th (1994, pH 6.7), and 13th (1996, pH 6.7) years of pH recovery are plotted. Sample sizes for Lake 223 varied from 115 to 1127 and for Lake 468 from 26 to 93 for individual years. The upper and lower limits of white sucker growth for years prior to pH decline in Lake 223, 1973–1976 (Mills et al. 1987), and the earliest years of Lake 468 data, 1973 and 1981, are also presented to facilitate comparisons among years.



in Lake 223 prior to acidification. In the same minnow traps that we caught fathead minnow in the stream between lakes 223 and 224, we caught brook stickleback. Therefore, we believe that some brook stickleback did emigrate from Lake 224 to Lake 223 each spring and these individuals may be the brood stock for the appearance of this species in our trap nets during pH recovery. Alternatively, the reported lowest pH for the occurrence of brook stickleback in lakes is approximately 4.6 (Wiener and Eilers 1987; Cusimano et al. 1990). This is well below pH 5.0, the lowest pH of Lake 223 during the acidification phase of the experiment. This species is present, but rarely caught, in many ELA lakes. Populations are usually found in the littoral zones of ELA lakes in emergent vegetation. A similar argument can be advanced for finescale dace. Finescale dace are usually rare in lakes that contain fathead minnow and pearl dace (Beamish et al. 1976; ELA staff, unpublished data). Finescale dace have a pH tolerance similar to that of pearl dace (Mills and Schindler 1986). Finescale dace are also present in upstream Lake 224 and possibly could have colonized Lake 223 during the final years of the acidification phase of the experiment. Although finescale dace were not caught in Lake 223 prior to 1980 and brook stickleback were not caught prior to 1984, we believe that a few individuals of each species were present in this lake prior to acidification, survived the acidification phase of the experiment, and increased recruitment during pH recovery so that each was caught frequently during pH recovery. Favorable food conditions during the initial years of pH recovery, such as an abundance of large *Daphnia* (Malley and Chang 1994), may have allowed the abundance of each species to increase. The brook stickleback may be an example of an opportunistic species, a situation well documented for acidic lakes, and these species can be very persistent during pH recovery (Nyberg 1984; Stenson et al. 1993).

Although fish recolonization of lakes recovering from acidification has been described for lakes other than Lake 223 (Kelso and Jeffries 1988; Appelberg et al. 1995), we do not believe that this will be commonplace in lakes recovering from acidification. The Lake 223 situation is unique; all other lakes near Lake 223 have neutral pH and healthy fish populations. Seed populations are very close to Lake 223 so that extremely good conditions existed for the recolonization of this lake. Other affected lakes may not have seed populations as conveniently located as those for Lake 223.

Pearl dace abundance

Our hypothesis of an increase in pearl dace abundance after recruitment resumed during pH recovery was confirmed. We were surprised that abundance of this species gradually

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decreased during the final years of pH recovery, but this may be related to competition for food resources with fathead minnow. In other ELA lakes where fathead minnow and pearl dace co-occur, pearl dace abundance is usually much lower than fathead minnow abundance (Beamish et al. 1976; ELA staff, unpublished data) and there is some dietary overlap between the two species at the ELA (Tallman et al. 1984). The inverse relationship between the abundance of these two species is very evident in the acidification and recovery phases of the Lake 223 experiment (Fig. 4). The abundance of pearl dace in Lake 223 is likely related to the reproductive success of fathead minnow.

Projections for future years

Although we have studied the recovery of Lake 223 fish populations from experimental acidification for 13 years, the recovery of fish populations in Lake 223 from the experimental acidification is not complete. Lake trout abundance is still much lower than that prior to acidification but may be increasing, as indicated by the 1996 estimate (Fig. 7). If abundance increases each year by the same number as the increase from 1995 to 1996, approximately 50 individuals, then it will take another 5 years for lake trout abundance to increase to 600 individuals, the number at the start of the acidification phase of the experiment. Low lake trout recruitment through many of the recovery years might be linked to the low abundance or absence of appropriate zooplankton prey species during the early life history of lake trout. *Daphnia galeata mendotae* was the most abundant daphnid in Lake 223 prior to acidification and during the years of high lake trout recruitment in the initial years of acidification. This species disappeared during the latter years of acidification but became extremely abundant during pH recovery starting in 1994 (D.F. Malley, ELA staff, unpublished data). Yearly variations in the cladoceran community structure and abundance can have a great influence on year-class strength of Arctic char (*Salvelinus alpinus*) (Næsje 1995). The same may be true for lake trout recruitment in Lake 223. If the large increase in *D. g. mendotae* continues, this may allow greater recruitment of lake trout year-classes in Lake 223. Similarly, fathead minnow larvae are present in lakes later in the year than pearl dace larvae (Scott and Crossman 1973). Age-0 lake trout might be too small to prey on pearl dace larvae early in the year but might be large enough to prey on fathead minnow larvae that occur later in the year.

Two important lake trout prey items, slimy sculpin and *M. relicta* (Nero and Schindler 1983), are still absent from Lake 223. Based on our experience with emigration of fathead minnow from Lake 224 to Lake 223, we believe that slimy sculpin may eventually colonize Lake 223. *Mysis relicta* is also present in upstream Lake 224, but this species may not readily colonize Lake 223 unless a very high spring runoff occurs so that the stream between the two lakes is very large and fast flowing. The absence of these two species from Lake 223 may be an important factor retarding the return of lake trout abundance to preacidification values.

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References

Allott, T.E.H., Harriman, R., and Battarbee, R.W. 1992. Reversibility of lake acidification at the Round Lock of Glenhead, Galloway, Scotland. Environ. Pollut. **77**: 219–225.

Appelberg, M., Lingdell, P.-E., and Andrén, C. 1995. Integrated studies of the effects of liming acidified waters (Iselaw-programme). Water Air Soil Pollut. **85**: 883–888.

Arnason, A.N., and Baniuk, L. 1978. POPAN-2: a data maintenance and analysis system for mark–recapture data. Charles Babbage Research Centre, St. Pierre, Man.

Bagenal, T.B., and Tesch, F.W. 1978. Age and growth. *In* Methods for assessment of fish production in freshwaters. 3rd ed. *Edited by* T.B. Bagenal. Blackwell Scientific Publications, Oxford, U.K. pp. 101–136.

Baker, J.P., Bernard, D.P., Christensen, S.W., Sale, M.J., Freda, J., Heltcher, K., Marmorek, D., Rowe, L., Scanlon, P., Suter, G., Warren-Hicks, W., and Welbourn, P. 1990. Biological effects of changes in surface water acid–base chemistry. NAPAP Rep. 13. *In* National Acid Precipitation Assessment Program, acidic deposition: state of science and technology. Vol. II. Government Printing Office, Washington, D.C.

Beamish, R.J., Blouw, L.M., and McFarlane, G.A. 1976. A fish and chemical study of 109 lakes in the Experimental Lakes Area (ELA), northwestern Ontario, with appended reports on lake whitefish ageing errors and the northwestern Ontario baitfish industry. Fish. Mar. Serv. Res. Dev. Tech. Rep. No. 607.

Chalanchuk, S.M. 1984. Aging a population of the white sucker, *Catostomus commersoni*, by the fin-ray method. Can. Tech. Rep. Fish. Aquat. Sci. No. 1321.

Chalanchuk, S.M., Mohr, L.C., and Allan, D.J. 1989. Ten years of data for four cyprinid species in Lake 114, an experimentally acidified lake in the Experimental Lakes Area, northwestern Ontario. Can. Data Rep. Fish. Aquat. Sci. No. 733.

Chalanchuk, S.M., Mohr, L.C., and Allan, D.J. 1991. Length data for four cyprinid species in Lake 302, a double-basin lake in the Experimental Lakes Area, northwestern Ontario. Can. Data Rep. Fish. Aquat. Sci. No. 846.

Chen, Y., and Harvey, H.H. 1995. Growth, abundance, and food supply of white sucker. Trans. Am. Fish. Soc. **124**: 262–270.

Cleugh, T.B., and Hauser, B.W. 1971. Results of the initial survey of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. **28**: 129–137.

Cook, R.B., Kelly, C.A., Schindler, D.W., and Turner, M.A. 1986. Mechanisms of hydrogen ion neutralization in an experimentally acidified lake. Limnol. Oceanogr. **31**: 134–148.

Cusimano, R.F., Baker, J.P., Warren-Hicks, W.J., Lesser, V., Taylor, W.W., Fabrizio, M.C., Hayes, D.B., and Baldigo, P.B. 1990. Fish communities in lakes in subregion 2B (Upper Peninsula of Michigan) in relation to lake acidity. EPA/600/3-89/021. Final Project Report. U.S. Environmental Protection Agency, Washington, D.C.

Ferreri, C.P., and Taylor, W.W. 1996. Compensation in individual growth rates and its influence on lake trout population dynamics

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in the Michigan waters of Lake Superior. J. Fish Biol. **49**: 763–777.

Gunn, J.M., and Keller, W. 1984. Spawning site water chemistry and lake trout (*Salvelinus namaycush*) sac fry survival during snowmelt. Can. J. Fish. Aquat. Sci. **41**: 319–329.

Gunn, J.M., and Keller, W. 1990. Biological recovery of an acid lake after reductions in industrial emissions of sulphur. Nature (Lond.), **345**: 431–433.

Havas, M., Woodfine, D.G., Lutz, P., Yung, K., MacIsaac, H.J., and Hutchinson, T.C. 1995. Biological recovery of two previously acidified, metal-contaminated lakes near Sudbury, Ontario, Canada. Water Air Soil Pollut. **85**: 791–796.

Haw, F., Bergman, P.K., Fralick, R.D., Buckley, R.M., and Blankenship, H.L. 1990. Visible implanted fish tag. Am. Fish. Soc. Symp. **7**: 311–315.

Healey, M.C. 1978. The dynamics of exploited lake trout populations and implications for management. J. Wildl. Manage. **42**: 307–328.

Healey, M.C. 1980. Growth and recruitment in experimentally exploited lake whitefish (*Coregonus clupeaformis*) populations. Can. J. Fish. Aquat. Sci. **37**: 255-267.

Johnson, L. 1976. The ecology of arctic populations of the lake trout, *Salvelinus namaycush*, lake whitefish, *Coregonus clupeaformis*, and Arctic char, *S. alpinus*, and associated species in unexploited lakes of the Canadian Northwest Territories. J. Fish. Res. Board Can. **33**: 2459–2488.

Jolly, G.M. 1965. Explicit estimates from capture–recapture data with both death and immigration — stochastic model. Biometrika, **52**: 225–247.

Keller, W., and Yan, N.D. 1998. Biological recovery from lake acidification: zooplankton communities as a model of patterns and processes. Restor. Ecol. **6**: 364–375.

Keller, W., Gunn, J.M., and Yan, N.D. 1992. Evidence of biological recovery in acid-stressed lakes near Sudbury, Canada. Environ. Pollut. **78**: 79–85.

Kelso, J.R.M., and Jeffries, D.S. 1988. Response of headwater lakes to varying atmospheric deposition in north-central Ontario, 1979-1985. Can. J. Fish. Aquat. Sci. **45**: 1905–1911.

Malley, D.F., and Chang, P.S.S. 1994. Assessing the health of a zooplankton community in a small Precambrian Shield lake during recovery from experimental acidification. J. Aquat. Ecosyst. Health, **3**: 273–286.

McCormick, J.H., Jensen, K.M., and Anderson, L.E. 1989. Chronic effects of low pH and elevated aluminum on survival, maturation, spawning and embryo–larval development of the fathead minnow in soft water. Water Air Soil Pollut. **43**: 293–307.

Mills, K.H., and Beamish, R.J. 1980. Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. Can. J. Fish. Aquat. Sci. **37**: 534–544.

Mills, K.H., and Chalanchuk, S.M. 1987. Population dynamics of lake whitefish (*Coregonus clupeaformis*) during and after the fertilization of Lake 226, the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. **44**: 55–63.

Mills, K.H., and Schindler, D.W. 1986. Biological indicators of lake acidification. Water Air Soil Pollut. **30**: 779–789.

Mills, K.H., Chalanchuk, S.M., Mohr, L.C., and Davies, I.J. 1987. Responses of fish populations in Lake 223 to 8 years of experimental acidification. Can. J. Fish. Aquat. Sci. **44**(Suppl. 1): 114–125.

Mills, K.H., Chalanchuk, S.M., Allan, D.J., and Mohr, L.C. 1995. Responses of lake whitefish (*Coregonus clupeaformis*) to exploitation at the Experimental Lakes Area, northwestern Ontario. Arch. Hydrobiol. Spec. Issues Adv. Limnol. **46**: 361–368.

Muniz, I.P., and Leivestad, H. 1980. Acidification — effects on freshwater fish. *In* Ecological Impact of Acid Precipitation, Proceedings of an International Conference, Sandefjord, Norway, March 11–14, 1980. *Edited by* D. Drablos and A. Tollan. SNSF Project, Oslo, Norway. pp. 84–92.

Næsje, T. 1995. Effects of introduced *Mysis relicta* on habitat utilization and feeding of Arctic char. Nord. J. Freshwater Res. **71**: 359–371.

Nero, R.W., and Schindler, D.W. 1983. Decline of *Mysis relicta* during the acidification of Lake 233. Can. J. Fish. Aquat. Sci. **40**: 1905–1911.

Nyberg, P. 1984. Impact of *Chaoborus* predation on planktonic crustacean communities in some acidified and limed forest lakes in Sweden. Inst. Freshwater Res. Drotttningholm Rep. **61**: 154–166.

Schindler, D.W., Mills, K.H., Malley, D.F., Findlay, D.L., Shearer, J.A., Davies, I.J., Turner, M.A., Linsey, G.A., and Cruikshank, D.R. 1985. Long-term ecosystem stress: the effects of experimental acidification on a small lake. Science (Washington, D.C.), **228**: 1395–1401.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Seber, G.A.F. 1982. Estimation of animal abundance and related parameters. 2nd ed. Macmillan Publishing Co., Inc., New York.

Stenson, J.A.E., Svensson, J.-E., and Cronberg, G. 1993. Changes and interactions in the pelagic community in acidified lakes in Sweden. Ambio, **22**: 277–282.

Tallman, R.F., Mills, K.H., and Rotter, R.G. 1984. The comparative ecology of pearl dace (*Semotilus margarita*) and fathead minnow (*Pimephales promelas*) in Lake 114, the Experimental Lakes Area, northwestern Ontario, with an appended key to the cyprinids of the Experimental Lakes Area. Can. MS Rep. Fish. Aquat. Sci. No. 1756.

Wiener, J.G., and Eilers, J.M. 1987. Chemical and biological status of lakes and streams in the Upper Midwest: assessment of acidic deposition effects. Lake Reservoir Manage. **3**: 365–378.

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Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon

Timothy E. Essington, Thomas P. Quinn, and Victor E. Ewert

Abstract: Individual female Pacific salmon fight for breeding space with conspecific and heterospecific females. We evaluated the consequences of this competition on the reproductive success of sockeye (*Oncorhynchus nerka*), chum (*Oncorhynchus keta*), and pink salmon (*Oncorhynchus gorbuscha*) in the Weaver Creek spawning channel, British Columbia. We hypothesized that differences in body size, relative abundance, and spawning date would influence the magnitude of interspecific interactions. Reproductive success (survival rate of eggs to emigrating fry) of the most abundant species, sockeye, was strongly and inversely correlated with conspecific abundance but not with the abundance of the other, less abundant species. Chum reproductive success was inversely correlated with sockeye abundance but not with the abundance of the scarce and smaller pink. Surprisingly, pink reproductive success was not correlated with sockeye abundance and only marginally correlated with chum abundance despite the fact that pink are smaller and spawn earlier than sockeye. Thus, intra- and inter-specific competition can substantially affect salmon reproductive success, but the magnitude of the competitive effects may depend on relative abundance, size, spawning date, and microhabitat preferences.

Résumé : Les femelles du saumon du Pacifique se battent avec les femelles de leur propre espèce ou d'autres espèces pour l'obtention d'un espace de frai. Nous avons évalué les conséquences de cette compétition sur le succès de la reproduction du saumon rouge (*Oncorhynchus nerka*), du saumon kéta (*Oncorhynchus keta*) et du saumon rose (*Oncorhynchus gorbuscha*) du chenal de frai du ruisseau Weaver, en Colombie-Britannique. Nous avons formulé l'hypothèse que des écarts ayant trait à la taille corporelle, à l'abondance relative et à la date du frai devraient influer sur l'importance des interactions interspécifiques. Le succès de la reproduction (taux de survie des oeufs jusqu'à l'alevin émergeant) de l'espèce la plus abondante, le saumon rouge, était fortement et inversement corrélé avec l'abondance des conspécifiques mais non avec l'abondance des individus des autres espèces, moins abondants. Le succès de la reproduction du saumon kéta était inversement corrélé avec l'abondance du saumon rouge mais non avec celle du saumon rose plus rare et plus petit. Au contraire des attentes, le succès de la reproduction du saumon rose n'était pas corrélé avec l'abondance du saumon rouge et ne l'était que très peu avec celle du saumon kéta, même si le saumon rose est plus petit et fraie plus tôt que le saumon rouge. Cette compétition intra et interspécifique peut modifier de façon appréciable le succès de la reproduction du saumon, mais l'importance des effets de la compétition peut être fonction de l'abondance relative, de la taille, de la date du frai et des préférences en matière de microhabitats.

[Traduit par la Rédaction]

Introduction

Competition for limited resources is one of the fundamental forms of behavioral and ecological interaction among organisms. Competitive behavior is often observed in habitat choice, foraging, or direct aggression and interference. However, the fitness consequences of competition may be difficult to detect, owing to insufficient or inaccurate data or confounding influences of density-independent processes. Interspecifc interactions, such as interference competition for breeding space (e.g., Breitburg 1987; Hayes 1987), may also obscure intraspecific competitive effects. Interspecific competition may be particularly intense for closely related species that share overlapping preferences for spawning sites (e.g., Ming and Noakes 1984). Typically, interspecific competition is inferred from aggressive behavior between species, but the fitness consequences of this competition are rarely demonstrated (Gustafsson 1987). As Thornhill (1987) pointed out, "...in order to determine whether interspecific interactions may affect the evolution of species differences and species coexistence, it is necessary to document not only that such interactions do occur but also that they have consequences for fitness."

Salmonid fishes provide excellent opportunities to study competition and its effects on fitness. Competition on the spawning grounds is widely accepted as an important part of the behavioral ecology of female salmon (e.g., Keenleyside and Dupuis 1988; Foote 1990) and the dynamics of salmon populations (e.g., McNeil 1969). This competition is a consequence of high densities of spawning fish on spawning grounds, particularly for some of the semelparous *Oncorhynchus* spp. Female salmon select, prepare, and defend the

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nest or redd site without assistance from males. Females are highly territorial, and territory holders resist displacement (Schroder 1982; McPhee and Quinn 1998) until they approach death, although size does provide some advantage in territory acquisition (van den Berghe and Gross 1989; Foote 1990). Thus, arrival date on the spawning grounds and, to some extent, body size affect territory acquisition. However, the duration of the breeding period of the population almost invariably exceeds the life span of individual females. Consequently, fertilized eggs deposited by an early-arriving female may be displaced by the digging of females superimposing their redds over the redd of the previous territory holder after her death. Such redd superimposition has been documented in many salmonid species (e.g., Witzel and MacCrimmon 1983; Hayes 1987; Beard and Carline 1991) but is believed to be a particularly important density-dependent mortality factor for pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) (McNeil 1969) and sockeye salmon (*Oncorhynchus nerka*) (Burgner et al. 1969). Large-bodied species should therefore affect the reproductive success of smaller-bodied species because larger females dig deeper redds (van den Berghe and Gross 1984; Kitano and Shimazaki 1995). In addition, early-spawning females should be more vulnerable to redd superimposition by conspecific (Essington et al. 1998; McPhee and Quinn 1998) and heterospecific individuals (Hayes 1987).

The shape of the spawner–recruit relationship often provides some insight into the mechanism of density dependence. An asymptotic (Beverton–Holt) spawner–recruit relationship reflects space limitation for spawning adults or offspring, while a dome-shaped (Ricker) spawner–recruit relationship reflects resource depletion by offspring. It is often difficult to distinguish these two models from a linear (density-independent) model or to distinguish between them based on empirical evidence for several reasons. First, the requisite long time series of data over a wide range of spawning population sizes are not common. Second, the number of females, their egg production, and the number of offspring must be accurately counted. Third, the importance of density-independent mortality factors such as flooding and freezing must be minimized.

In this study, we investigate the relative importance of intra- and inter-specific competition on the reproductive success of sockeye, chum, and pink salmon spawning in the Weaver Creek spawning channel (WCSC). The WCSC is a controlled-flow environment with ideal gravel and relatively uniform habitat, limiting the confounding influences of density-independent factors and habitat preference on recruitment. The three species of salmon differ in body size (pink being the smallest and chum the largest) and spawning date in this system (pink spawning somewhat earlier than chum and sockeye), two factors that we predicted would affect interspecific competition. The WCSC has recorded the number of returning females, fecundity, egg deposition, and fry production for over 30 years, allowing us to assess the extent of competitive interactions by fitting stock–recruit models and to identify whether density dependence occurs prior to or after egg deposition. We used these data to test whether less numerous species are primarily affected by the density of the most numerous species and whether larger-bodied and later-spawning species are least affected by interspecific competition.

Materials and methods

Site description

The WCSC is located on Weaver Creek, a small tributary of the Harrison River, in the Fraser River system, British Columbia, approximately 100 km upstream from the mouth of the Fraser River and 2 km downstream of Harrison Lake. The WCSC was constructed in 1965 to augment sockeye production, which had been declining, apparently due to habitat degradation. It was operated and maintained by the International Pacific Salmon Fisheries Commission until 1985, after which it was operated by the Department of Fisheries and Oceans.

Data collection

The channel, 2930 m long by 6.1 m wide, was designed to provide optimal conditions for survival of developing embryos and hatchling alevins prior to emergence as free-swimming fry (Rosberg et al. 1986). The channel is operated to receive a discharge of 0.55 $m^3 \cdot s^{-1}$, with a resulting mean depth of 0.25 m and mean velocity of 0.4 $m \cdot s^{-1}$. Spawning substrate consists of 1.2- to 10.2-cm-diameter gravel, approximately 40 cm deep. Periodic mechanical cleaning of the gravel and a settling pond upstream minimize siltation that might otherwise reduce water and oxygen flow to embryos in the gravel. Egg to fry survival in the spawning channel is approximately an order of magnitude greater than in Weaver Creek proper (Rosberg et al. 1986).

The number of spawning females of each species entering the spawning channel is controlled at a downstream weir to achieve an abundance of approximately 19 000 female sockeye plus approximately 2000 combined female chum and pink. Pink were present only during odd-numbered years. Sockeye are preferentially admitted into the spawning channel (relative to their abundance in the creek), and the ratio of pink to chum to sockeye averaged 2:5:32 over the odd-numbered years of the study. From 1965 to 1997 the number, species, and sex of returning fish were recorded. From 1975 to 1997, mean fecundity of sockeye and pink was also assessed by sampling 50 (sockeye) or 25 individuals (pink) each year. Prior to 1975, fecundity was estimated from regression of fecundity and standard length (Rosberg et al. 1986). For chum, fecundity was measured in 25 individuals each year from 1989 to 1997, and the mean value from this period (2793) was used for all other years. Mean female body size of carcasses and of fish sacrificed for fecundity estimates was measured from the posterior edge of the orbit of the eye to the hypural plate.

Total egg deposition for each species was calculated by estimating potential egg deposition (number of females times mean fecundity) and subtracting the number of eggs retained in females, estimated by inspection of female carcasses. Each carcass was classified as 100% spawned, 50% spawned, or 0% spawned, and the number of eggs retained by females was measured in a subsample of each category. Thus, total egg retention was equal to the egg retention in each category times the number of females in each category. For chum, data were not available on degree of spawning for 1965–1981, so potential egg deposition could not be adjusted for unspawned eggs for these years.

The number of fry produced in the channel was estimated by using a trap at the end of the channel. The trap sampled 5% of the fry migrating over a weir. The species composition of a subsample of the catch was determined each night and the nightly total was estimated from the weights of the catch and the weights of fry of each species. Because sockeye fry greatly outnumbered chum and pink fry, estimates of chum and pink fry abundance are less precise than those for sockeye.

Data analysis

To compare the timing of spawning for each species, we calculated the cumulative proportion of females entering the channel

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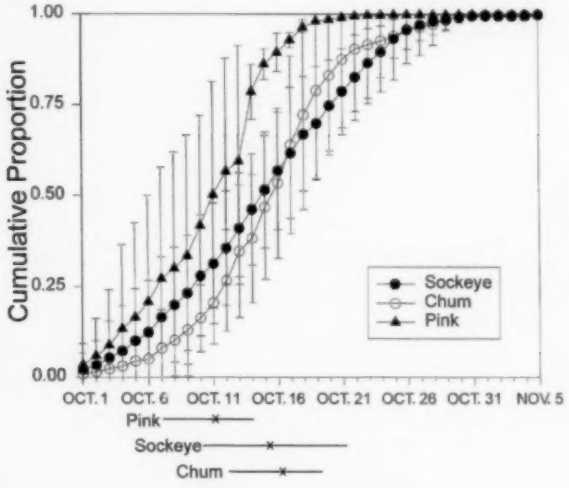
Fig. 1. Temporal pattern of channel entry for female sockeye, chum, and pink salmon. Data points represent the mean (±SD) cumulative proportion of the total run entering the channel on each day, averaged over 5 years. Lines at the bottom indicate the peak entry period, and X indicates the 50% entry date for each species



each day, averaged across years (1993–1997), and used two parameters to describe these data. The first was the date at which 50% of the annual total number of females had entered the channel (median entry date). The second was the peak entry period, defined by the dates when 25–75% of the annual total entered the channel. These dates will closely approximate the peak spawning dates because most female salmon spawn within 1–3 days of entry onto spawning grounds (Schroder 1982; Quinn and Foote 1994; McPhee and Quinn 1998). Ideally, we would evaluate the entry date of each species for each year to determine how interannual variation in entry date affects interspecific competition, but the limited data on channel entry (1993–1997) preclude this analysis.

To evaluate whether each species exhibited density-dependent reproductive success, we fit three stock–recruit curves for each species (Ricker 1975). The general form of the stock–recruit curves was

$$(1) \qquad R = f(P)e^{V}$$

where R is the number of fry produced, P is the number of females, and V is an error term that is normally distributed with mean 0 and variance σ^2, i.e., we assumed lognormal errors. The stock–recruit function, $f(P)$, was either a linear (density independent), Beverton–Holt (density dependent, saturation curve), or Ricker curve (density dependent, dome-shaped curve).

Linear:

$$(2a) \qquad f(P) = \alpha P$$

Beverton–Holt:

$$(2b) \qquad f(P) = \frac{1}{\alpha + \beta P^{-1}}$$

Ricker:

$$(2c) \qquad f(P) = \alpha P \exp(-\beta P).$$

Best-fitting model parameters were those that minimized the negative log-likelihood of the residuals. Instead of including σ^2 as a free parameter, we calculated the likelihood profile by estimating σ^2 from the model (Hilborn and Mangel 1997). To determine whether there was significant density-dependent reductions in reproductive success, we compared the likelihoods of the linear model with that of the Beverton–Holt or the Ricker model. For this analysis, we recognized that both the Ricker and Beverton–Holt models could be reduced to the linear model by setting one parameter to zero (α in the Beverton–Holt model, β in the Ricker model). Thus, the linear models were nested within the Beverton–Holt or Ricker models, allowing us to compare models using the likelihood ratio (Hilborn and Mangel 1997). For this test, the more complex model (Beverton–Holt or Ricker) provided a significantly better fit to the data than the linear model if the ratio of likelihoods exceeded 7.25 (or equivalently, if the difference in negative log likelihoods exceeded 1.98).

For those species that exhibited density-dependent reproductive success, we evaluated whether density dependence occurred primarily by affecting the behavior of mature females or by affecting the survivorship of deposited eggs. We first determined whether the proportion of females that were 100% spawned was correlated with female abundance. We then evaluated whether the postdeposition reproductive success (fry produced per deposited eggs) was correlated with female abundance. For all analyses, we used simple linear regression to identify significant correlations. We used standard diagnostics of residuals to evaluate whether the assumptions of normality and linearity were met (Draper and Smith 1981).

To evaluate the effect of heterospecifics on reproductive success, we determined whether the total reproductive success of each species was correlated with heterospecific abundance. Total reproductive success was defined as the ratio of fry produced to potential egg deposition. We used stepwise multiple regression to identify significant correlations between total reproductive success and conspecific and heterospecific abundances. Although we were not specifically interested in the effect of conspecifics in this analysis, it was necessary to include this parameter as a covariate. We set α for entry and removal from the model equal to 0.05. When we identified a significant correlation, we identified whether density-dependent reduction in reproductive success occurred prior to or after egg deposition by determining whether the proportion of females that spawned and (or) the postdeposition reproductive success were correlated with heterospecific abundance.

Results

Pink entered the channel earliest in each year and had the earliest 50% entry date (October 11). Timing of returns was similar between chum and sockeye, with sockeye having a slightly earlier median entry date (October 15) than chum (October 16). However, sockeye had longer peak entry period (11 days) than pink and chum (both 7 days). Consequently, peak entry period for sockeye broadly overlapped with the peak period of pink and chum and overlapped the 50% entry date of both species (Fig. 1). Moreover, the peak entry period of sockeye extended later than that of pink and chum. Peak entry periods of pink and chum overlapped by 3 days, yet did not overlap with the other species' 50% entry date. These differences were consistent across years, except for 1994 when sockeye 50% entry date occurred 5 days later than chum 50% entry date.

Data on mean female size were available for 1993, 1995, and 1997 for pink and for 1989–1997 for sockeye and chum (except for 1994 when no data were available for chum). During these years, pink had the smallest mean size (430 mm), with annual means ranging from 424 to 437 mm. Sockeye were larger than pink, with a mean size of 502 mm and annual

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Table 1. Number of returning salmon females, egg deposition, and fry production in the WCSC, 1965–1995.

	Sockeye				Chum				Pink			
Year	Females	Potential egg deposition (millions)	Actual egg deposition (millions)	No. of fry (millions)	Females	Potential egg deposition (millions)	Actual egg deposition (millions)	No. of fry (millions)	Females	Potential egg deposition (millions)	Actual egg deposition (millions)	No. of fry (millions)
1965	2 986	11.90	11.44	9.00	487	1.36	na	0.98	32	0.07	0.06	0.04
1966	3 424	14.75	14.13	12.39	92	0.26	na	0.23				
1967	1 631	6.85	6.08	4.50	202	0.56	na	0.47	70	0.16	0.14	0.11
1968	784	3.18	2.95	2.56	1138	3.18	na	2.70				
1969	9 671	37.59	36.33	32.62	1286	3.59	na	3.23	108	0.22	0.21	0.19
1970	2 519	10.75	9.89	8.19	482	1.35	na	1.24				
1971	1 520	6.35	6.29	4.51	696	1.94	na	1.76	178	0.36	0.35	0.24
1972	6 418	25.61	25.29	15.21	7333	20.48	na	12.51				
1973	10 857	46.64	45.74	35.05	3077	8.59	na	4.29	364	0.70	0.69	0.54
1974	15 044	67.28	59.21	36.85	2893	8.08	na	6.18				
1975	10 287	43.79	41.64	25.68	2532	7.07	na	6.24	633	1.28	1.23	0.39
1976	16 525	69.11	65.09	52.75	6322	17.66	na	5.86				
1977	19 172	83.32	77.72	19.52	2486	6.94	na	1.65	600	1.28	1.19	0.28
1978	17 527	76.68	75.15	40.98	3535	9.87	na	2.39				
1979	11 424	48.85	47.90	26.67	2753	7.69	na	3.06	444	0.81	0.79	0.11
1980	24 043	96.99	94.72	52.60	1905	5.32	na	1.87				
1981	10 244	42.10	41.56	21.62	2928	8.18	na	2.81	671	1.31	1.30	0.26
1982	30 983	130.50	122.60	56.05	3160	8.83	8.13	3.67				
1983	12 037	52.19	51.19	29.31	2927	8.18	8.04	4.43	1030	1.86	1.85	1.17
1984	25 098	101.35	97.51	45.40	2186	6.11	5.96	1.69				
1985	11 362	47.61	45.37	18.88	2812	7.85	7.81	1.98	2297	4.63	4.60	0.92
1986	24 375	94.53	80.57	19.14	333	0.93	0.72	0.23				
1987	18 281	80.99	77.65	33.77	509	1.42	1.38	0.77	660	1.32	1.29	0.81
1988	14 192	57.73	56.63	20.44	404	1.13	1.07	0.58				
1989	7 527	27.97	27.65	21.66	1570	4.48	4.40	0.93	1326	2.37	2.34	0.92
1990	6 061	21.76	21.59	18.22	1426	3.87	3.82	1.97				
1991	15 563	56.66	55.70	42.90	263	0.70	0.68	0.36	1156	1.80	1.78	1.48
1992	18 080	64.81	63.91	46.09	681	1.76	1.73	1.39				
1993	17 354	62.41	61.66	38.82	1366	3.46	3.40	2.67	1823	2.77	2.73	1.36
1994	17 301	67.79	67.36	42.22	1297	3.59	3.48	2.74				
1995	11 486	32.73	31.68	21.96	1360	4.14	3.93	2.50	1077	1.77	1.60	0.85
1996	20 120	68.53	67.83	45.05	1315	3.88	3.835	1.85				
1997	10 700	38.44	32.03	13.70	1567	4.74	4.707	3.31	1497	2.29	2.24	0.97
Mean	12 867	51.45	49.15	27.71	1919	5.37	3.94	2.68	821	1.47	1.43	0.62

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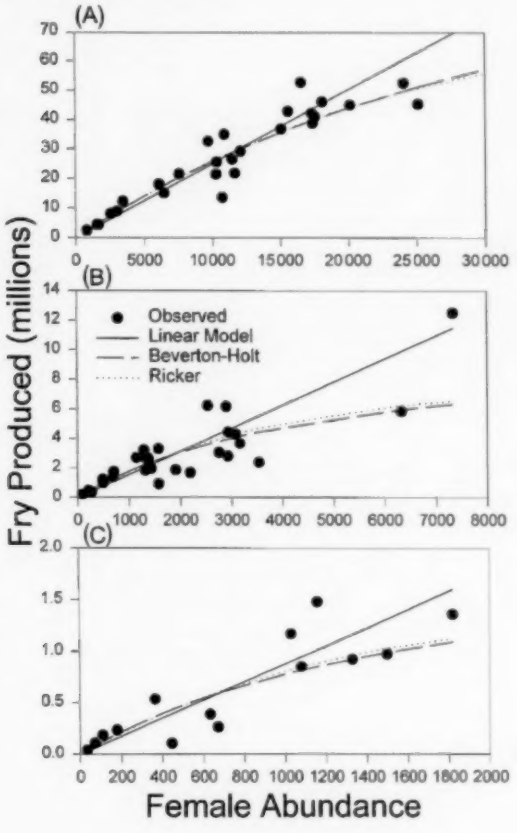
Fig. 2. Best-fitting stock–recruit curves fit to (A) sockeye, (B) chum, and (C) pink salmon data. For each species, a linear (density independent), Beverton–Holt (density dependent), and Ricker (density dependent) curve were fit to the data. See Table 2 for parameter estimates.



means ranging from 483 to 518 mm. Chum had the largest mean length, 587 mm, ranging from 558 to 610 mm.

Female sockeye abundance varied greatly, from 784 in 1968 to 30 983 in 1982 (Table 1). Sockeye fry production was similarly variable, ranging from 2.56 million in 1968 to 52.75 million in 1976. Abundance of female chum and pink also varied greatly, ranging from 92 to 7333 (chum) and from 32 to 2297 (pink) (Table 1). Fry production ranged from 0.23 to 12.51 million for chum and from 0.04 to 1.17 million for pink.

A large flood occurred in 1977 and an outbreak of infectious haematopoietic necrosis virus occurred from 1985 to 1988 (Traxler and Rankin 1989). Because reproductive success was markedly reduced during these years owing to density-independent effects, data from these years were not used for any analyses (which estimate density-dependent processes). For sockeye, the Beverton–Holt and Ricker models both provided a significantly better fit to the data than the linear model (Fig. 2), with a likelihood ratio of 190 for the Beverton–Holt/linear comparison and 176 for the Ricker/linear comparison (Table 2). Both of these ratios greatly exceeded the critical value of 7.25 needed for statistical significance at the 0.05 level. Assuming a uniform, noninformative prior probability for each model (i.e., each model was equally likely, a priori), then the best-fitting Beverton–Holt model was only 1.08 times as likely as the best-fitting Ricker model based on the ratio of likelihoods. The similarity in the likelihoods of these two models is also indicated by the similarity in the shapes of the two curves (Fig. 2). Thus, there was strong evidence for density-dependent reproductive success for sockeye, but we could not ascertain whether the Beverton–Holt or the Ricker model best described this density dependence.

There was also some evidence for density-dependent reproductive success for chum (Fig. 2). Both the Ricker and Beverton–Holt models fit the data significantly better than the linear model, with a likelihood ratio of 26.2 for the Beverton–Holt/linear comparison and 12.9 for the Ricker/linear comparison (Table 2). The Beverton–Holt model was 2.0 times as likely as the Ricker model, but both model fits were strongly influenced by two high-leverage data points, corresponding to chum abundances of 6322 and 7333 (Fig. 2). Excluding either of these points resulted in large changes in the model fits because there were few data points within that range of female abundances. Thus, the evidence for density-dependent reproductive success was equivocal.

There was no evidence of density-dependent reproductive success for pink. Neither the Beverton–Holt model (likelihood ratio = 2.6) nor the Ricker model (likelihood ratio = 2.17) provided a significantly better fit to the data than the linear model (Table 2). These likelihood ratios were substantially smaller than those calculated from the sockeye and chum data.

Because sockeye showed evidence for density-dependent reproductive success, we evaluated at what life stage density dependence took place. There was no relationship between the total number of females and mean fecundity ($F_{1,31} < 0.001$, $P = 0.99$) (data not shown) or the proportion of females that spawned ($F_{1,31} = 0.362$, $P = 0.552$) (Fig. 3). However, there was a strong negative correlation between postdeposition reproductive success and the abundance of spawning females ($R^2 = 0.34$, $F_{1,26} = 14.848$, $P < 0.001$) (Fig. 3). Thus, density-dependent reduction in reproductive success occurred after fertilization and was manifest by reduced survivorship of deposited eggs. The slope of this relationship was -1.06×10^{-5}, indicating a 10% decline in postdeposition reproductive success with every 10 000 sockeye females. Although proportional data are often transformed to meet the assumption of normality, residual analyses indicated that no transformation was needed.

The three species exhibited different responses to heterospecific abundances. Stepwise multiple regression of sockeye total reproductive success against sockeye, chum, and pink abundance indicated that only conspecific abundance affected sockeye reproductive success ($R^2 = 0.28$, $F_{1,26} = 11.46$, $P = 0.002$). In other words, there was no evidence of competitive effects of pink or chum on sockeye reproductive success. Chum reproductive success was best predicted by a model that included only sockeye abundance ($R^2 = 0.285$, $F_{1,26} = 13.7$, $P = 0.008$) (Fig. 4). The slope of this relationship was slightly steeper than that observed for the intraspecific density-dependent effects on sockeye (slope = -1.73×10^{-5}), but the standard errors of these parameter estimates overlapped broadly. Chum abundance was not a

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Table 2. Parameter estimates and negative log-likelihoods (L) of best-fitting stock–recruit models for each salmon species.

	Linear		Beverton–Holt			Ricker		
Species	α	L	α	β	L	α	β	L
Sockeye	2.5 × 10–3	–1.7	6.9 × 10–3	318	–6.98*	3.2 × 10–3	1.7 × 10–5	–6.90*
Chum	1.6 × 10–3	15.39	9.4 × 10–2	466	12.56*	1.9 × 10–3	1.1 × 10–4	13.26*
Pink	8.8 × 10–4	10.63	4.6 × 10–1	828	10.64	1.1 × 10–3	3.3 × 10–4	10.82

Note: Recruits expressed as millions of fry produced. An asterisk denotes a significantly better fit than the linear model ($\alpha = 0.05$).

good predictor of chum total reproductive success when sockeye abundance was included in the model, suggesting that there was little intraspecific effect on chum reproductive success. There was no correlation between the proportion of chum females that spawned and sockeye abundance (R^2 = 0.00) (Fig. 4). We did not regress postdeposition reproductive success versus sockeye abundance because removal of data points from the infectious haematopoietic necrosis virus years left only nine data points and insufficient statistical power. However, because there was little evidence that sockeye affected chum by reducing their egg deposition, we can infer that sockeye most likely affected chum by reducing the postdeposition reproductive success.

Total reproductive success of pink was best predicted by a model that included only chum female abundance (R^2 = 0.31, $F_{1,15} = 8.2$, $P = 0.012$) (Fig. 5). This reduction was not attributable to a reduction in the proportion of spawning females, as there was no relationship between this parameter and chum abundance ($F_{1,14} = 0.827$, $P = 0.379$) (Fig. 5B). However, there was a significant reduction in postdeposition reproductive success attributable to chum abundance (R^2 = 0.34, $F_{1,15} = 9.27$, $P = 0.008$) (Fig. 5C). The slope of this line was steep (slope = -1.44×10^{-4}) and was nearly an order of magnitude larger than the effect of sockeye on sockeye and chum postdeposition reproductive success. Further, the 95% confidence interval for the slope (-1.87×10^{-4}, -0.93×10^{-4}) did not overlap with the 95% confidence interval of the slopes of the sockeye postdeposition reproductive success versus sockeye spawner abundance (-1.34×10^{-5}, -0.79×10^{-5}) or the chum–sockeye regression (-2.19×10^{-5}, -0.13×10^{-5}).

Discussion

Based on species-specific patterns of relative abundance, body size, and timing of salmon in WCSC, we predicted that sockeye (the most numerous species) would experience the greatest intraspecific competition whereas pink (the smallest, scarcest, and earliest spawning species) would experience the greatest interspecific competition. Because of their relative abundance, pink should have little effect on the reproductive success of either chum or sockeye whereas sockeye should have the greatest effect on heterospecific reproductive success. Chum, being the largest species, should not be affected by the smaller, earlier-spawning, and generally scarce pink. Chum should also be too scarce to display intraspecific effects, but they might be affected by sockeye, which were much more abundant but are smaller and spawn slightly earlier than chum.

Consistent with the predictions, sockeye were the only species to experience any intraspecific competition, and they were not affected by heterospecifics. The Ricker and Beverton–Holt spawner–recruit models were three orders of magnitude more likely than the linear model, clearly indicating that reproductive success was reduced at high densities. Further, linear regression predicted that mean sockeye reproductive success is reduced 50% at high spawner densities. As predicted, pink had no effect on the reproductive success of sockeye or chum, most likely because they were too small, too scarce, and spawn too early to dig up many redds of the other species. Chum, despite being larger than sockeye, were affected by them, presumably because sockeye were very numerous and the timing of spawning overlapped. Chum recruitment was slightly correlated with conspecific abundance, but this correlation vanished after accounting for the effects of sockeye. The lack of significant density dependence likely reflects the fact that chum abundance was, on average, one sixth that of sockeye.

Contrary to our expectations, pink were unaffected by the abundance of sockeye ($F_{1,15} = 0.847$, $P = 0.372$). Because of their early spawning date and small size, we expected pink to be highly susceptible to redd superimposition and experience large reductions in reproductive success as a function of sockeye and, to a lesser extent, chum abundance. However, there was no correlation between pink reproductive success and sockeye abundance. There are several plausible explanations for this result. One possibility is that our statistical power was too small to detect significant effects. Pink were only present during odd-numbered years; thus, there were only half as many observations as for the other species. However, this explanation seems unlikely. Because WCSC is operated to provide ideal physical habitat, variation in reproductive success due to abiotic factors is minimal. Moreover, there was a fourfold range in sockeye abundance, providing a more than adequate range to detect significant correlations. Thus, the lack of statistical significance likely reflects an absence of ecologically significant effects.

One explanation for the absence of a negative interaction between pink and sockeye is that there are isolating mechanisms that reduce interspecific interactions. For example, differences in habitat preferences may give rise to spatial segregation of spawning sites. Because these species differ in mean body size and egg size, their optimal spawning habitats may also differ. Fukushima and Smoker (1998) reported that pink and sockeye exhibited only small differences in microhabitat preferences, yet spawned in very different areas of Lake Creek, Alaska. Alternatively, size-mediated aggression between females may result in spatial segregation of redd sites. Territoriality in spawning salmonids, especially females, is common and territorial disputes are typically won by larger females (Foote 1990), especially if neither is in possession of the territory. Further, the sockeye females were always more abundant than pink females, even early in the spawning period when pink channel entries were at their

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Fig. 3. Effect of female sockeye salmon abundance on the (A) proportion of sockeye females that spawned and (B) postdeposition reproductive success (fry produced per deposited eggs). There was a significant reduction in postdeposition reproductive success with increasing sockeye abundance but no effect of sockeye abundance on the proportion of females that spawned.

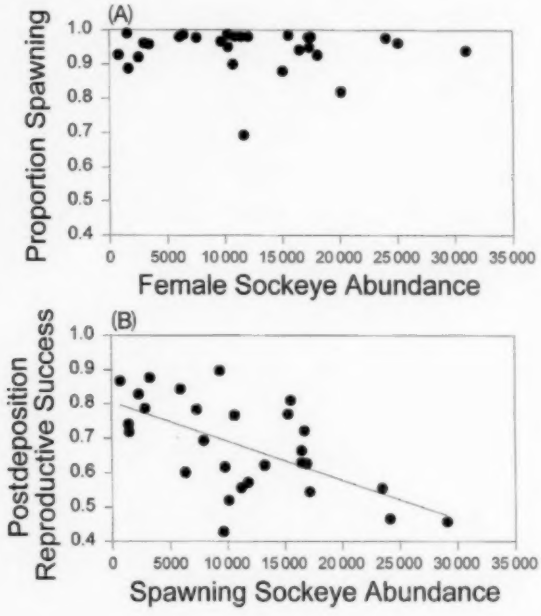
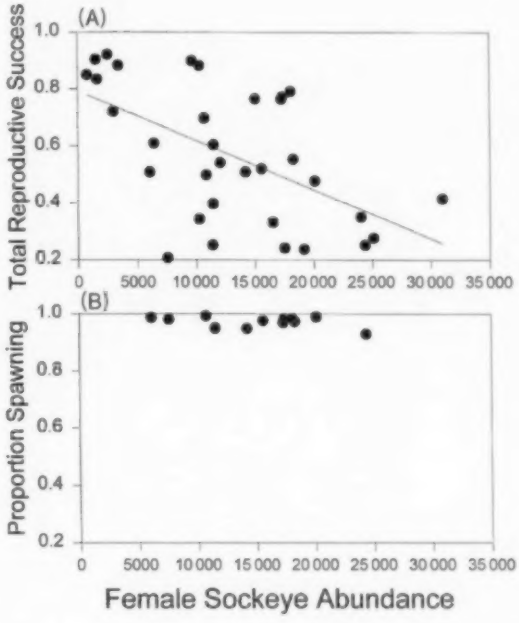


Fig. 4. Effect of female sockeye salmon abundance on (A) chum salmon total reproductive success and (B) proportion of female chum that were 100% spawned. There was a significant reduction between chum total reproductive success and sockeye abundance but no effect of sockeye on the proportion of chum that spawned.



peak. Thus, all three species might seek the same habitat, but the larger chum and sockeye might displace pink into less desirable habitat. Because these habitats are less likely to be used by later-spawning chum or sockeye, they are less susceptible to redd superimposition.

Both hypotheses predict nonrandom distributions of the species throughout the channel. Although designed to provide uniformly suitable habitat, the channel cannot be homogeneous. There are edges and a thalweg in the channel, and there are deeper, slower sections associated with its 180° bends and low, in-channel structures built to increase oxygenation, in addition to the long stretches of more uniform depth typical of most of the channel. It was beyond the scope of this study to document the spatial distribution of the species, but behavioral observations of hundreds of courting pairs of salmon have indicated that pink and chum tend to build redds in the vicinity of conspecifics and that interspecific aggression among females is common (Quinn 1999). Those spatial distribution data are consistent with both hypotheses whereas the aggression data are more consistent with the second hypothesis. Under the first hypothesis, reproductive success of all species would be similar, whereas under the second, the average reproductive success of pink might be lower than that of the other species if they were forced to use inferior habitat. Although there were differences in total reproductive success between pink (mean = 0.51), sockeye (mean = 0.61), and chum (mean = 0.57), these differences were not significant (ANOVA of arcsine square root transformed data, $F_{1,63} = 2.6$, $P = 0.11$). Moreover, Fukushima and Smoker (1998) found no evidence that pink redd site selection was influenced by sockeye abundance. Thus, the hypothesis that differences in body size resulted in habitat segregation appears unlikely.

Even with rather long and very accurate data sets, it was difficult to distinguish between Ricker and Beverton–Holt patterns of density dependence. That is, it was unclear whether fewer (Ricker) or the same (Beverton–Holt) number of recruits were produced at high densities as were produced at intermediate adult densities. This partly reflects the limitations of these data; the number of females entering the channel is controlled to avoid overcrowding, so we do not have fry production data for very high spawner abundances. However, it is interesting that we failed to find evidence to support a dome-shaped spawner–recruit relationship over an asymptotic curve despite a 40-fold range of sockeye spawner abundance. This suggests that if density dependence is of the Ricker type, it is only severe enough to cause a dome-shaped spawner–recruit curve at very high spawner abundances.

The importance of intra- and inter-specific competition in Pacific salmon species will vary from site to site and from year to year according to the relative abundance of salmon and the amount of suitable habitat available. Even so, this study under controlled conditions indicates the potential for interspecific competition among sockeye and chum. It also suggests that the fitness consequences of the competition observed in Weaver Creek result primarily from redd superimposition. The mechanisms and potential for interspecific competition identified in Weaver Creek should generalize to

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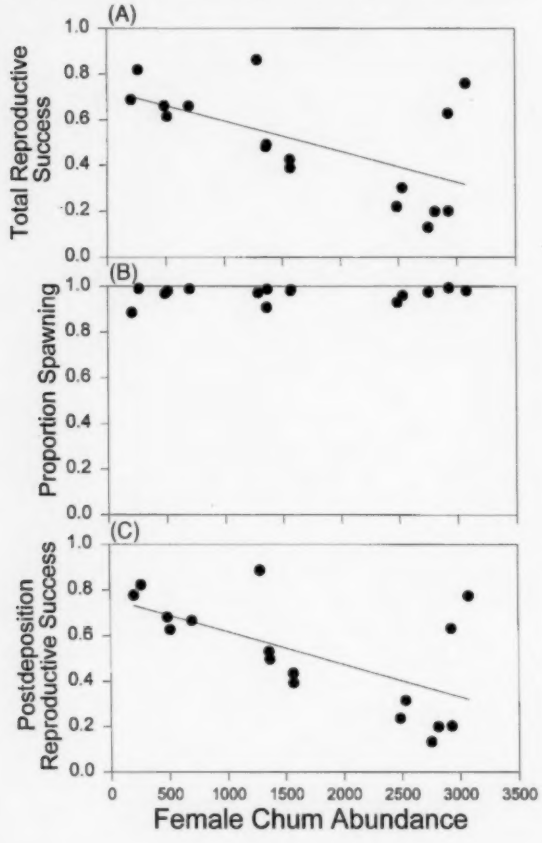
Fig. 5. Effect of female chum salmon abundance on (A) pink salmon total reproductive success, (B) proportion of female pink that were 100% spawned, and (C) pink postdeposition reproductive success. Total reproductive success and postdeposition reproductive success were reduced at increasing chum abundances, but the proportion spawning was unaffected by chum abundance.



other natural situations, provided the spawning habitat and densities are not too artificial. Female salmon density at WCSC averaged 0.83 female·m^{-2} during 1965–1997. This density exceeds the 0.5 female·m^{-2} that Burgner et al. (1969) concluded was the general carrying capacity for Bristol Bay stream spawning areas. However, the WCSC density is not unprecedented for wild populations. Densities of 0.7 and 0.8 female·m^{-2} were documented by Quinn and Foote (1994) and McPhee and Quinn (1998), respectively, in two sockeye populations in Alaska. Fleming and Gross (1989) documented female coho salmon (*Oncothunchus kisutch*) density in 11 northwestern streams and reported a mean density in suitable spawning habitat of 0.72 female·m^{-2}. Thus, the processes operating in the channel may be comparable with those in other areas of high-quality habitat and high salmon densities.

The survival rates from egg deposition to emergence were considerably higher than typical survival rates in natural channels. Rosberg et al. (1986) reported that egg to fry survival rates in the spawning channel are approximately an order of magnitude greater in Weaver Creek proper. Foerster (1968) reviewed survival to emergence studies on sockeye and concluded that 10% survival was typical (versus 57–69% in this study). Survival to emergence of chum averaged 14% in the Fraser River (Beacham and Starr 1982), 22% prior to logging in Carnation Creek (Scrivener and Brownlee 1989), and 5.6% in Hooknose Creek (Hunter 1959). Heard (1991) concluded that "...survival of pink salmon from egg to alevin, even in highly productive streams, commonly reaches only 10–20%...." Presumably, the lower survival rates in natural systems, compared with the WCSC, result from such factors as floods that scour the gravel and damage or dislodge embryos (McNeil 1966; Seegrist and Gard 1972; Thorne and Ames 1987), poor circulation from low flow and small substrate size (Chapman 1988), low dissolved oxygen (McNeil 1966), and predation (Hunter 1959; Phillips and Claire 1966), all of which are minimized in the WCSC. Although these factors affect the survival of embryos in natural systems, they do not preclude the effects of competition. Rather, they are additional effects and also serve to mask the effects of competition by introducing variability into data sets.

We believe that interspecific competition among adults can be an important factor in salmon population dynamics in the many cases of sympatric spawning. Spawning date may dictate the direction of asymmetric competitive effects by facilitating disturbance of one species' redds by species spawning afterward. However, these effects may be mitigated by differences in habitat use. The Pacific salmon species have ranges that overlap broadly but do not coincide (Groot and Margolis 1991), presumably for a variety of reasons. Within the area of overlap, few streams support all species. Some patterns of habitat use are clearly related to the species-specific feeding and migration patterns of juveniles, e.g., the affinity of sockeye for streams flowing into lakes and coho for streams with pools and complex channel structure (Groot and Margolis 1991). In other cases, salmon body size or swimming performance may be matched to the depth and velocity of the river. At a still finer scale, patterns of microhabitat preference (e.g., depth, velocity, substrate, cover) may segregate the species. It is unclear to what extent this segregation reflects past competition and present preferences.

In summary, competitive interactions had profound effects on the population-level reproductive success of sympatric salmonids. In the WCSC, sockeye and chum reproductive success was reduced by 50% in response to variations in sockeye abundance. However, the magnitude of interspecific effects may depend on relative body size, spawning date, and habitat use patterns within the spawning grounds as a whole. Interspecific competition by adults probably has very important effects on the dynamics and evolution of salmon populations, although these effects are hard to document owing to the many other factors affecting habitat use by adults and survival to emergence of their progeny.

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References

Beacham, T.D., and Starr, P. 1982. Population biology of chum salmon, *Oncorhynchus keta*, from the Fraser River, British Columbia. Fish. Bull. U.S. **80**: 813–825.

Beard, T.D., and Carline, R.F. 1991. Influence of spawning and other stream habitat features on spatial variability of brown trout. Proc. Am. Fish. Soc. **120**: 711–722.

Breitburg, D.L. 1987. Interspecific competition and the abundance of nest sites: factors affecting sexual selection. Ecology, **68**: 1844–1855.

Burgner, R.L., DiCostanzo, C.J., Ellis, R.J., Harry, G.Y., Jr., Hartman, W.L., Kerns, O.E., Jr., Mathisen, O.A., and Royce, W.F. 1969. Biological studies and estimates of optimum escapements of sockeye salmon in major river systems in southwestern Alaska. Fish. Bull. U.S. **67**: 405–459.

Chapman, D.W. 1988. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc. **117**: 1–21.

Draper, N.R., and Smith H. 1981. Applied regression analysis. John Wiley & Sons, New York.

Essington, T.E., Sorensen, P.W., and Paron, D.G. 1998. High frequency of redd superimposition of brook trout and brown trout in a Minnesota stream cannot be explained by habitat availability alone. Can. J. Fish. Aquat. Sci. **55**: 2310–2316.

Fleming, I.A., and Gross, M.R. 1989. Evolution of adult female life history and morphology in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution, **43**: 141–157.

Foerster, R.E. 1968. The sockeye salmon, *Oncorhynchus nerka*. Bull. Fish. Res. Board Can. No. 162.

Foote, C.J. 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. Behaviour, **115**: 283–314.

Fukushima, M., and Smoker, W.W. 1998. Spawning habitat segregation of sympatric sockeye and pink salmon. Trans. Am. Fish. Soc. **127**: 253–260.

Groot, C., and Margolis, L. (*Editors*). 1991. Pacific salmon life histories. University of British Columbia Press, Vancouver, B.C.

Gustafsson, L. 1987. Interspecific competition lowers fitness in collared flycatchers, *Ficedula albicollis*: an experimental demonstration. Ecology, **68**: 291–296.

Hayes, J.W. 1987. Competition for spawning space between brown trout (*Salmo trutta*) and rainbow trout (*S. gairdneri*) in a lake inlet tributary, New Zealand. Can. J. Fish. Aquat. Sci. **44**: 40–47.

Heard, W.R. 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*). *In* Pacific salmon life histories. *Edited by* C. Groot and L. Margolis. University of British Columbia Press, Vancouver, B.C. pp. 121–230.

Hilborn, R., and Mangel, M. 1997. The ecological detective: confronting models with data. Princeton University Press, Princeton, N.J.

Hunter, J.G. 1959. Survival and production of pink and chum salmon in a coastal stream. J. Fish. Res. Board Can. **16**: 835–886.

Keenleyside, M.H., and Dupuis, H.M.C. 1988. Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. **66**: 262–265.

Kitano, S., and Shimazaki, K. 1995. Spawning habitat and nest depth of female Dolly Varden *Salvelinus malma* of different body size. Fish. Sci. **61**: 776–779.

McNeil, W.J. 1966. Effect of the spawning bed environment on reproduction of pink and chum salmon. Fish. Bull. U.S. **64**: 495–523.

McNeil, W.J. 1969. Survival of pink and chum salmon eggs and alevins. *In* Symposium on Salmon and Trout in Streams. *Edited by* T.G. Northcote. H.R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. pp. 101–117.

McPhee, M.V., and Quinn, T.P. 1998. Factors affecting the duration of nest defense and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. Environ. Biol. Fishes, **51**: 369–375.

Ming, F.W., and Noakes, D.L.G. 1984. Spawning site selection and competition in minnows (*Pimephales notatus* and *P. promelas*: Pisces, Cyprinidae). Biol. Behav. **9**: 227–234.

Phillips, R.W., and Claire, E.W. 1966. Intragravel movement of the reticulate sculpin, *Cottus perplexus*, and its potential as a predator on salmonid embryos. Trans. Am. Fish. Soc. **95**: 210–212.

Quinn, T.P. 1999. Variation in Pacific salmon reproductive behaviour associated with species, sex and levels of competition. Behaviour, **136**: 179–204.

Quinn, T.P., and Foote, C.J. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon (*Oncorhynchus nerka*). Anim. Behav. **48**: 751–761.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Rosberg, G.E., Scott, K.J., and Rithaler, R. 1986. Review of the International Pacific Salmon Fisheries Commission's sockeye and pink salmon enhancement facilities on the Fraser River. Department of Fisheries and Oceans, Salmonid Enhancement Program, Vancouver, B.C.

Schroder, S.L. 1982. The influence of intrasexual competition on the distribution of chum salmon in an experimental stream. *In* Proceedings of the Salmon and Trout Migratory Symposium. *Edited by* E.L. Brannon and E.O. Salo. University of Washington Press, Seattle, Wash. pp. 275–285.

Scrivener, J.C., and Brownlee, M.J. 1989. Effects of forest harvesting on spawning gravel and incubation survival of chum (*Oncorhynchus keta*) and coho salmon (*O. kisutch*) in Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. **46**: 681–696.

Seegrist, D.W., and Gard, R. 1972. Effects of floods on trout in Sagehen, California. Trans. Am. Fish. Soc. **101**: 478–482.

Thorne, R.E., and Ames, J.J. 1987. A note on variability of marine survival of sockeye salmon (*Oncorhychus nerka*) and effects of flooding on spawning success. Can. J. Fish. Aquat. Sci. **44**: 1791–1795.

Thornhill, R. 1987. The relative importance of intra- and interspecific competition in scorpionfly mating systems. Am. Nat. **130**: 711–729.

Traxler, G.S., and Rankin, J.B. 1989. An infectious hematopoietic necrosis epizootic in sockeye salmon, *Oncorhynchus nerka*, in Weaver Creek spawning channel, Fraser River system, B.C., Canada. Dis. Aquat. Org. **6**: 221–226.

van den Berghe, E.P., and Gross, M.R. 1984. Female size and nest depth in coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **41**: 204–206.

van den Berghe, E.P., and Gross, M.R. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution, **43**: 125–140.

Witzel, L.D., and MacCrimmon, H.R. 1983. Redd site selection by brook trout and brown trout in southwestern Ontario streams. Trans. Am. Fish. Soc. **112**: 760–771.

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Demographic analysis of gummy shark (*Mustelus antarcticus*) and school shark (*Galeorhinus galeus*) off southern Australia by applying a generalized Lotka equation and its dual equation

Yongshun Xiao and Terence I. Walker

Abstract: Although Lotka's equation is commonly used for calculating the intrinsic rate of increase with time of a fish population in demographic analysis, its dual equation has never been derived. In this paper, we establish an explicit relationship between the intrinsic rate of increase with time of a fish population and its instantaneous rate of natural mortality from an age-dependent population dynamics model, derive a generalized Lotka equation for calculating the intrinsic rate of increase with time, and derive its dual equation for calculating the intrinsic rate of decrease with age. The virginal intrinsic rate of increase with time of the gummy shark (*Mustelus antarcticus*) population was calculated as 0.115957·year^{-1} and its intrinsic rate of decrease with age as –0.312957·year^{-1}. The virginal intrinsic rate of increase with time of the school shark (*Galeorhinus galeus*) population was calculated as 0.109480·year^{-1} and its intrinsic rate of decrease with age as –0.216980·year^{-1}. The generalized Lotka equation and its dual equation thus derived imply that both reproductive schedules of a population of animals and its instantaneous rate of total mortality determine its intrinsic rate of increase with time, whereas its reproductive schedules alone determine its intrinsic rate of decrease with age.

Résumé : Bien que l'équation de Lotka soit communément utilisée pour calculer le taux intrinsèque d'accroissement en fonction du temps des populations de poissons dans les analyses démographiques, son équation duale n'a jamais été établie. Dans le présent article, nous établissons une relation explicite entre le taux d'accroissement en fonction du temps d'une population de poissons et son taux instantané de mortalité naturelle à partir d'un modèle de dynamique de population fondé sur l'âge, une équation généralisée de Lotka pour calculer le taux intrinsèque d'accroissement en fonction du temps, et son équation duale pour calculer le taux intrinsèque de décroissance en fonction de l'âge. Le taux intrinsèque d'accroissement en fonction du temps d'une population vierge de chien de mer arctique, *Mustelus antarcticus*, a été établi à 0,115957·an^{-1}, et son taux intrinsèque de décroissance en fonction de l'âge à –0,312957·an^{-1}. Le taux intrinsèque d'accroissement en fonction du temps d'une population vierge de requin hâ, *Galeorhinus galeus*, a été établi à 0,109480·an^{-1}, et son taux intrinsèque de décroissance en fonction de l'âge à –0,216980·an^{-1}. L'équation généralisée de Lotka et l'équation duale qui en a été tirée impliquent que les calendriers de reproduction d'une population animale et son taux instantané de mortalité totale déterminent ensemble son taux intrinsèque d'accroissement en fonction du temps, alors que seuls ses calendriers de reproduction déterminent son taux intrinsèque de décroissance en fonction de l'âge.

[Traduit par la Rédaction]

Introduction

Demographic analysis has been performed for several groups of animals, notably chondrichthyans and mammals (Jones and Geen 1977; Hoenig and Gruber 1990; Hoff and Musick 1990; Woodley and Read 1991; Cailliet 1992; Cailliet et al. 1992; Cortés 1995, 1998, 1999; Cortés and Parsons 1996; Smirkey and Musick 1996; Au and Smith 1997; Márquez-Farias and Castillo-Géniz 1998; Márquez-Farias et al. 1998; Smith et al. 1998). This is partly because the intrinsic rate of (natural) increase with time of an animal (referred to below as fish) population is a measure of its reproductive potential and hence its resilience to human exploitation (Roff 1981; Smith et al. 1998). In fact, Smith et al. (1998) have computed the intrinsic rates of increase with time of 26 shark species for a hypothesized instantaneous rate of total mortality of twice its corresponding rate of natural mortality and used them fruitfully as a measure of the relative ability of different species to recover from fishing pressure. Shark populations are usually amenable to demographic analysis for three main reasons: (*i*) they are often widely and thinly distributed, with poorly reported catches and uncertain estimates of fishing effort, (*ii*) their vital rates are often difficult to estimate because of ageing and sampling difficulties, and (*iii*) most of them do not have strongly varying strengths of year-classes, thereby having a relatively

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temporally stable age distribution (a usually made assumption in demographic analysis) (Smith et al. 1998).

Lotka's (1922) equation is one of the most commonly used models for calculating the intrinsic rate of increase with time of a fish population for performing demographic analysis. However, a literature survey indicates that there is still a need to make explicit some of its many subtleties, particularly those in the fisheries context. Also, the equation is so elegant in functional form and so central to demography that its dual equation must be of great interest to biologists in general and demographers in particular and should be derived. Indeed, if its dual equation exists and is deducible, both equations will complement each other in improving the theory of demographic analysis and in characterizing the dynamics of fish populations. Moreover, the instantaneous rate of natural mortality of a fish population has long been suspected to be related to its intrinsic rate of increase (Au and Smith 1997). Since both are vital population characteristics but both are relatively difficult to determine, such a relationship, if derived, will allow one to be calculated from the other.

Gummy shark (*Mustelus antarcticus*) and school shark (*Galeorhinus galeus*) (sensu Last and Stevens 1994) are major species in the Australian southern shark fishery. The fishery extends from Western Australia in the west through South Australia to Bass Strait (Victoria) and Tasmania in the east and has an annual landed value of A$16 million (Walker et al. 1997). From the beginning of this fishery in the mid-1920s, shark catches increased steadily with the use of bottom-set longlines until the early 1970s. Since then, most sharks have been taken by bottom-set gill nets mainly of 6-in. mesh size in Bass Strait, beyond which school shark have been harvested by gill nets mainly of 7-in. mesh size. From 1973 to 1997, the annual catch of gummy shark ranged from 1208 to 2181 t in live weight in Bass Strait; for all of southern Australia, annual catch ranged from 1652 to 3408 t for gummy shark and from 1040 to 3018 t for school shark. Unlike many other species of shark, both gummy and school shark are intensively studied and their stocks have been assessed by use of various production models (Walker 1992, 1994, 1998).

In this paper, we establish an explicit relationship between the intrinsic rate of increase of a fish population and its instantaneous rate of natural mortality from an age-dependent population dynamics model, derive a generalized Lotka (1922) equation for calculating the intrinsic rate of increase with time, derive its dual equation for calculating the intrinsic rate of decrease with age, and apply that relationship and both equations to data on gummy shark in Bass Strait and school shark off southern Australia.

Materials and methods

Model

Let $N(a,t) \geq 0$, $-\infty < a_0 \leq a < \infty$, $-\infty < t_0 \leq t < \infty$ denote the number of fish of age a at time t in a population of females, with the average age at birth a_0 and reference time t_0. The change in $N(a,t)$ in a small time interval $[t, t + \Delta t]$ of length Δt is assumed to satisfy the familiar first-order partial differential equation (e.g., Xiao 1997, pp. 21–25)

$$(1) \quad \lim_{\Delta t \to 0} \frac{N(a + \Delta a, t + \Delta t) - N(a,t)}{\Delta t} \approx \alpha \frac{\partial N(a,t)}{\partial a} + \frac{\partial N(a,t)}{\partial t} = -Z(a,t)N(a,t)$$

where $\alpha = \frac{da}{dt}$ is the derivative of age a with respect to time t ($\alpha = 1$ if both age a and time t have the same units, e.g., years), $Z(a,t) = F(a,t) + M(a,t) \geq 0$ is the instantaneous rate of total (fishing $F(a,t)$ and natural $M(a,t)$) mortality and can capture the effects of fish age, time, and density dependence, $\frac{\partial N(a,t)}{\partial a}$ is the absolute rate of change of $N(a,t)$ with respect to age a, and $\frac{\partial N(a,t)}{\partial t}$ is the absolute rate of change of $N(a,t)$ with respect to time t. Equation 1 describes the dynamics of a closed (to immigration, emigration, and any other processes that change $N(a,t)$) population. For useful perspectives on age-dependent models in nonfisheries contexts, readers may wish to consult Lotka (1922, 1924), McKendrick (1926), Kermack and McKendrick (1927, 1932), and Hoppensteadt (1975).

Equation 1 can be written as

$$(2) \quad \frac{\alpha}{N(a,t)} \frac{\partial N(a,t)}{\partial a} + \frac{1}{N(a,t)} \frac{\partial N(a,t)}{\partial t} = -Z(a,t)$$

where $\frac{1}{N(a,t)} \frac{\partial N(a,t)}{\partial a}$ is the relative (per capita) rate of change of $N(a,t)$ with respect to age a and $\frac{1}{N(a,t)} \frac{\partial N(a,t)}{\partial t}$ is the relative rate of change of $N(a,t)$ with respect to time t.

Equation 2 can be rewritten as

$$(3) \quad \alpha \Lambda(a,t) + \lambda(a,t) = -Z(a,t)$$

where $\lambda(a,t) = \frac{1}{N(a,t)} \frac{\partial N(a,t)}{\partial t}$ is the well-known intrinsic rate of (natural) increase of $N(a,t)$ with time t because $N(a,t)$ increases with time (unless the population concerned remains unchanged in size with respect to time t or is to collapse to extinction); by analogy, $\Lambda(a,t) = \frac{1}{N(a,t)} \frac{\partial N(a,t)}{\partial a}$ is the intrinsic rate of (natural) decrease of $N(a,t)$ with age a because $N(a,t)$ decreases with age from natural and fishing mortalities (unless the population concerned remains unchanged in size with respect to age a).

Equation 3, rewritten as $-\alpha\Lambda(a,t) = \lambda(a,t) + Z(a,t)$, makes clear a remarkable fact that $-\alpha\Lambda(a,t)$ is the instantaneous rate of total or gross productivity of fish of age a at time t, $\lambda(a,t)$ is the instantaneous rate of net productivity of fish of age a at time t, and $Z(a,t)$ is the instantaneous rate of productivity of fish of age a at time t lost due to total mortality. At the population level at time t, for an age group at all times, and at the population level at all times, eq. 3 becomes, respectively

$$(3.1) \quad \alpha \int_{a_0}^{\infty} \Lambda(a,t)\,da + \int_{a_0}^{\infty} \lambda(a,t)\,da = -\int_{a_0}^{\infty} Z(a,t)\,da \Rightarrow \alpha\Lambda(t) + \lambda(t) = -Z(t)$$

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$$(3.2)\quad \alpha\int_{t_0}^{\infty}\Lambda(a,t)\,dt+\int_{t_0}^{\infty}\lambda(a,t)\,dt=-\int_{t_0}^{\infty}Z(a,t)\,dt\Rightarrow\alpha\Lambda(a)+\lambda(a)=-Z(a)$$

$$(3.3)\quad \alpha\int_{t_0}^{\infty}\int_{a_0}^{\infty}\Lambda(a,t)\,da\,dt+\int_{t_0}^{\infty}\int_{a_0}^{\infty}\lambda(a,t)\,da\,dt=-\int_{t_0}^{\infty}\int_{a_0}^{\infty}Z(a,t)\,da\,dt\Rightarrow\alpha\Lambda+\lambda=-Z.$$

Thus, eq. 2 implies, and eqs. 3–3.3 actually give explicitly, some long-suspected fundamental relationships in fisheries science between two well-known quantities, i.e., the intrinsic rate of increase of a population and its instantaneous rate of natural mortality. Such explicit relationships make it simple to calculate any of their quantities from the others. They also provide an upper bound for the instantaneous rate of fishing, natural, or total mortality: $0 \le Z(a,t) \le -\alpha\Lambda(a,t)$ if $\lambda(a,t) \ge 0$; $0 \le F(a,t) \le -\alpha N(a,t)$; and $0 \le M(a,t) \le -\alpha\Lambda(a,t)$; $Z(a,t) = -\alpha\Lambda(a,t)$ only if the population concerned is at its temporal equilibrium (i.e., $\lambda(a,t) = 0$).

The rate of birth of females, or the number of females born at age a_0, by all females at time t is

$$(4)\quad N(a_0,t)=\int_{a_0}^{\infty}R(a,t)\beta(a,t)N(a,t)\,da,\qquad t\ge t_0$$

where $0 \le R(a,t) \le 1$ is the average proportion of the progeny that are female produced by all female fish of age a at time t and $\beta(a,t) \ge 0$ is the average reproductive rate of all female fish of age a at time t. Both $R(a,t)$ and $\beta(a,t)$ can capture the effects of fish age, time, and density dependence. The rate of birth of males by all females at time t is

$$N_m(a_0,t)=\int_{a_0}^{\infty}[1-R(a,t)]\beta(a,t)N(a,t)\,da,\qquad t\ge t_0\ .$$

The age distribution of a population of female fish is said to be stable over time t, when $N(a,t)$ can be written as a product of an age-dependent function $A(a)$ and a time-dependent function $T(t)$, i.e.:

$$(5)\quad N(a,t)=A(a)T(t).$$

The assumption of stable age distribution implies that the proportion of the number of fish of age a, or within any age bracket $[a_1,a_2]$, $a_0 \le a_1 < a_2 < \infty$, in a population of females at time t does not change with time t. To see its logic, notice that

$$(5.1)\quad \int_{a_1}^{a_2}N(a,t)\,da=T(t)\int_{a_1}^{a_2}A(a)\,da$$

$$(5.2)\quad \int_{a_0}^{\infty}N(a,t)\,da=T(t)\int_{a_0}^{\infty}A(a)\,da.$$

Division of both sides of eq. 5 by those of eq. 5.2 and division of both sides of eq. 5.1 by those of eq. 5.2 yield

$$p(a)=\frac{N(a,t)}{\int_{a_0}^{\infty}N(a,t)\,da}=\frac{A(a)}{\int_{a_0}^{\infty}A(a)\,da}$$

$$\frac{\int_{a_1}^{a_2}N(a,t)\,da}{\int_{a_0}^{\infty}N(a,t)\,da}=\frac{\int_{a_1}^{a_2}A(a)\,da}{\int_{a_0}^{\infty}A(a)\,da}=\int_{a_1}^{a_2}p(a)\,da$$

both of which are functions of age a only and are independent of time t, as they should be. The mean $\mu(a - a_0)$ and variance $\sigma^2(a - a_0)$ of ages (from birth at age a_0) of all individuals in the population with a stable age distribution are then given, respectively, by

$$\mu(a-a_0)=\int_{a_0}^{\infty}(a-a_0)p(a)\,da$$

$$\sigma^2(a-a_0)=\int_{a_0}^{\infty}[a-a_0-\mu(a-a_0)]^2\,p(a)\,da,$$

$$-\infty < a_0 \le a < \infty$$

Many other statistics can be calculated of ages (from birth at age a_0) of all individuals in the population with a stable age distribution.

Under the assumption of stable age distribution:

$$\frac{\partial N(a,t)}{\partial a}=T(t)\frac{dA(a)}{da}$$

$$\frac{\partial N(a,t)}{\partial t}=A(a)\frac{dT(t)}{dt}$$

$$\frac{1}{N(a,t)}\frac{\partial N(a,t)}{\partial a}=\frac{1}{A(a)}\frac{dA(a)}{da}$$

$$\frac{1}{N(a,t)}\frac{\partial N(a,t)}{\partial t}=\frac{1}{T(t)}\frac{dT(t)}{dt}$$

which imply that the relative rate of change of $N(a,t)$ with respect to age a is equal to that of $A(a)$ with respect to age a; the relative rate of change of $N(a,t)$ with respect to time t is equal to that of $T(t)$ with respect to time t such that

$$(6)\quad \frac{\alpha}{A(a)}\frac{dA(a)}{da}+\frac{1}{T(t)}\frac{dT(t)}{dt}=-Z(a,t).$$

Equations 5 and 6 run into difficulties, however, because each contains two undetermined functions: $A = A(a)$ and $T = T(t)$. For this assumption (eq. 5) and its resulting equation (eq. 6) to work, both $A = A(a)$ and $T = T(t)$ must be determined uniquely by making an additional assumption. Now, we consider only two of the numerous assumptions that can be made.

If $\frac{1}{T(t)}\frac{dT(t)}{dt}=\lambda$ is a constant and $Z(a,t) = Z(a)$ is a function of age a only, eq. 6 can be written as a system of two ordinary differential equations:

$$(7)\quad \frac{1}{T(t)}\frac{dT(t)}{dt}=\lambda$$

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$$\frac{\alpha}{A(a)}\frac{dA(a)}{da} = -\lambda - Z(a)$$

solution of which as an initial value problem, with $T(t)\big|_{t=t_0} = T(t_0)$ and $A(a)\big|_{a=a_0} = A(a_0)$, yields

$$T(t) = T(t_0)\, e^{\lambda(t-t_0)} \tag{8}$$

and

$$A(a) = A(a_0)\, e^{-\frac{\lambda}{\alpha}(a-a_0)-\frac{1}{\alpha}\int_{a_0}^{a} Z(s)\,ds}$$

substitution of which into eq. 5 gives

$$N(a,t) = A(a_0)T(t_0)\, e^{\lambda(t-t_0)-\frac{\lambda}{\alpha}(a-a_0)-\frac{1}{\alpha}\int_{a_0}^{a} Z(s)\,ds} \tag{9}$$

$$= N(a_0,t)\, e^{-\frac{\lambda}{\alpha}(a-a_0)-\frac{1}{\alpha}\int_{a_0}^{a} Z(s)\,ds}$$

substitution of which into eq. 4, and notice that $N(a_0, t) = A(a_0)T(t_0)\, e^{\lambda(t-t_0)}$, give

$$1 = \int_{a_0}^{\infty} R(a,t)\beta(a,t)\, e^{-\frac{\lambda}{\alpha}(a-a_0)-\frac{1}{\alpha}\int_{a_0}^{a} Z(s)\,ds}\, da, \qquad t \geq t_0. \tag{10}$$

If $R(a,t) = R$ = constant, eq. 10 becomes

$$\frac{1}{R} = \int_{a_0}^{\infty} \beta(a,t)\, e^{-\frac{\lambda}{\alpha}(a-a_0)-\frac{1}{\alpha}\int_{a_0}^{a} Z(s)\,ds}\, da, \qquad t \geq t_0. \tag{11}$$

Equation 10 or 11 is the generalized Lotka (1922) equation. Now, λ can be determined from reproductive schedules $R(a,t)$ and $\beta(a,t)$ and total mortality $Z(a,t) = Z(a)$. After calculating λ, one can use eq. 3 or 6 to calculate $\Lambda(a,t) = \Lambda(a)$.

Because, from eq. 3, $\alpha\Lambda(a) + \lambda = -Z(a)$ or $\Lambda(a) = -[\lambda + Z(a)]/\alpha$, eqs. 10 and 11 can be rewritten, respectively, as

$$1 = \int_{a_0}^{\infty} R(a,t)\beta(a,t)\, e^{\int_{a_0}^{a} \Lambda(s)\,ds}\, da, \qquad t \geq t_0 \tag{12}$$

$$\frac{1}{R} = \int_{a_0}^{\infty} \beta(a,t)\, e^{\int_{a_0}^{a} \Lambda(s)\,ds}\, da, \qquad t \geq t_0. \tag{13}$$

Now, $\Lambda(a,t) = \Lambda(a)$ can be determined from reproductive schedules $R(a,t)$ and $\beta(a,t)$ alone. After calculating $\Lambda(a)$, one can use eq. 3 or 6 to calculate $\lambda(a,t) = \lambda$.

Thus, the generalized Lotka (1922) equation (eq. 10 or 11) for calculating the intrinsic rate of increase with time and eq. 12 or 13 for calculating the intrinsic rate of decrease with age form a duality, complement each other in improving the theory of demographic analysis and in characterizing the dynamics of fish populations, and are related to each other through a simple relationship (eq. 3 or 6).

If $\frac{1}{A(a)}\frac{dA(a)}{da} = \Lambda$ is a constant and $Z(a,t) = Z(t)$ is a function of time t only, eq. 6 can be written as a system of two ordinary differential equations

$$\frac{1}{A(a)}\frac{dA(a)}{da} = \Lambda \tag{14}$$

and

$$\frac{1}{T(t)}\frac{dT(t)}{dt} = -\alpha\Lambda - Z(t)$$

solution of which as an initial value problem, with $T(t)\big|_{t=t_0} = T(t_0)$ and $A(a)\big|_{a=a_0} = A(a_0)$, yields

$$A(a) = A(a_0)\, e^{\Lambda(a-a_0)} \tag{15}$$

and

$$T(t) = T(t_0)\, e^{-\alpha\Lambda(t-t_0)-\int_{t_0}^{t} Z(s)\,ds}$$

substitution of which into eq. 5 gives

$$N(a,t) = A(a_0)T(t_0)\, e^{\Lambda(a-a_0)-\alpha\Lambda(t-t_0)-\int_{t_0}^{t} Z(s)\,ds} \tag{16}$$

$$= N(a_0,t)\, e^{\Lambda(a-a_0)}$$

substitution of which into eq. 4, and notice that $N(a_0, t) = \Lambda\ (a_0)\ T(t_0) e^{-\alpha\Lambda(t-t_0)-\int_{t_0}^{t} Z(s)\,ds}$, give

$$1 = \int_{a_0}^{\infty} R(a,t)\beta(a,t)\, e^{\Lambda(a-a_0)}\, da, \qquad t \geq t_0. \tag{17}$$

If $R(a,t) = R$ = constant, eq. 17 becomes

$$\frac{1}{R} = \int_{a_0}^{\infty} \beta(a,t)\, e^{\Lambda(a-a_0)}\, da, \qquad t \geq t_0. \tag{18}$$

Now, Λ in eq. 17 or 18 can be determined from reproductive schedules $R(a,t)$ and $\beta(a,t)$ alone. After determining Λ, one can use eq. 3 or 6 to calculate $\lambda(a,t) = \lambda(t)$.

Because, from eq. 3, $\alpha\Lambda + \lambda(t) = -Z(t)$ or $\Lambda = -[\lambda(t) + Z(t)]/\alpha$, eqs. 17 and 18 can be rewritten, respectively, as

$$1 = \int_{a_0}^{\infty} R(a,t)\beta(a,t)\, e^{-\frac{1}{\alpha}\{\lambda(t)+Z(t)\}(a-a_0)}\, da, \qquad t \geq t_0 \tag{19}$$

$$\frac{1}{R} = \int_{a_0}^{\infty} \beta(a,t)\, e^{-\frac{1}{\alpha}\{\lambda(t)+Z(t)\}(a-a_0)}\, da, \qquad t \geq t_0. \tag{20}$$

Equation 19 or 20 is the generalized Lotka (1922) equation. Now, $\lambda(a,t) = \lambda(t)$ can be determined from reproductive schedules $R(a,t)$ and $\beta(a,t)$ and total mortality $Z(a,t) = Z(t)$. After calculating $\lambda(t)$, one can use eq. 3 or 6 to calculate $\Lambda(a,t) = \Lambda$.

Thus, the generalized Lotka (1922) equation (eq. 19 or 20) for calculating the intrinsic rate of increase with time and eq. 17 or 18 for calculating the intrinsic rate of decrease with age form a duality, complement each other from a different angle (age or time) in improving the theory of demographic analysis and in characterizing the dynamics of fish populations, and are related to each other through a simple relationship (eq. 3 or 6).

Under the assumption of stable age distribution, the temporally stable age distribution of a fish population depends only on the intrinsic rate of decrease with age or on gross productivity, so that

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$$(21)\quad p(a)=\frac{e^{\int_{a_0}^{a}\Lambda(s)\,ds}}{\int_{a_0}^{\infty}e^{\int_{a_0}^{a}\Lambda(s)\,ds}\,da}$$

with the mean $\mu(a-a_0)$ and variance $\sigma^2(a-a_0)$ of ages (from birth at age a_0) of all individuals in the population being given, respectively, by

$$\mu(a-a_0)=\int_{a_0}^{\infty}(a-a_0)p(a)\,da$$

$$\sigma^2(a-a_0)=\int_{a_0}^{\infty}[a-a_0-\mu(a-a_0)]^2\,p(a)\,da,$$

$$-\infty<a_0\le a<\infty$$

Equation 21 implies that the population concerned can have a stable age distribution for an infinite number of values of fishing mortality $F(a,t)$ subject to the constraint of eq. 3 or 6. If $\Lambda(a)=\Lambda=$ constant, then $p(a)$ becomes the probability density function of the exponential distribution, with parameter $-\Lambda$, so that

$$p(a)=-\Lambda\,e^{\Lambda(a-a_0)},\ -\infty\le a_0\le a<\infty$$

with the mean $\mu(a-a_0)$ and variance $\sigma^2(a-a_0)$ of ages (from birth at age a_0) of all individuals in the population being given, respectively, by $\mu(a-a_0)=-\frac{1}{\Lambda}$ and $\sigma^2(a-a_0)=\frac{1}{\Lambda^2}$.

Data analysis

Equation 10 or 11 and eq. 12 or 13 can be solved, respectively, for the intrinsic rate of increase with time λ and intrinsic rate of decrease with age $\Lambda(a)$. For this purpose, all other quantities, including the average proportion of the progeny that are female produced by all female fish of age a at time t, $R(a,t)$, age-specific reproductive rate, $\beta(a,t)$, total mortality, $Z(a,t)=Z(a)$, and their associated quantities, must be specified.

The sex ratio of both species of shark at birth is assumed to be 1:1, or $R(a,t)=1/2$, because that of their in utero embryos was observed to be 1:1 (Walker 1992). Because age a and time t have the same units (years), $\alpha=1$. Pups of both species of shark are born at age $a_0=0$ (Walker 1992).

Age-specific reproductive rate $\beta(a,t)$ of gummy shark is a product of the mean number of births annually per pregnant female shark of age a (assumed to be the number of embryos carried by the pregnant shark of age a) and the proportion of female sharks of age a that become pregnant each year (Walker 1994), i.e.,

$$\beta(a,t)=\begin{cases}0 & a_0\le a<a_m\\ e^{-1.8520+0.0032L(a)}\times\left(\dfrac{0.6060}{1+e^{11.7310-0.0092L(a)}}\right) & a_m\le a<\infty\end{cases}$$

where $a_m=3.9694$ years is the average age at first maturity and $L(a)$ is the total length of a shark of age a satisfying the familiar von Bertalanffy growth equation $L(a)=L_\infty(1-e^{-K(a-a_0')})$, where $L_\infty=2019$ mm, $K=0.1230\cdot\text{year}^{-1}$, and $a_0'=-1.55$ years (Moulton et al. 1992). For school shark,

$$\beta(a,t)=\begin{cases}0 & a_0\le a<a_m\\ [-42.93+0.0468L(a)]\times\left(\dfrac{0.3333}{1+e^{34.9006-0.0235L(a)}}\right) & a_m\le a<\infty\end{cases}$$

where $a_m=11.2404$ years is the average age at first maturity and $L(a)$ is the total length of a shark of age a satisfying the von Bertalanffy growth equation, where $L_\infty=1618.3$ mm, $K=0.1600\cdot\text{year}^{-1}$, and $a_0'=-1.28$ years (Moulton et al. 1992).

The instantaneous rate of total mortality $Z(a)$ is the sum of the instantaneous rate of natural mortality $M(a)$ and the instantaneous rate of fishing mortality $F(a)$. The $M(a)$ was estimated at $0.1970\cdot\text{year}^{-1}$ for gummy shark (Walker 1992) and $0.1075\cdot\text{year}^{-1}$ for school shark (Grant et al. 1979). In our calculations, we also assumed that $F(a)=F=$ constant for both species of shark. Thus, one can calculate λ by use of eq. 10 or 11 for a given value of F (subject, of course, to the constraint of eq. 3 or 6) and values of other quantities and then calculate $\Lambda(a)$ by use of eq. 3 or 6. Indeed, because λ, M, and F are constants, $\Lambda(a)=\Lambda=-(\lambda+F+M)/\alpha$ is a constant, so that one can also determine Λ by use of eq. 12 or 13 without first specifying the value of F and then calculate λ by use of eq. 3 or 6 for any value of F (subject, again, to the constraint of eq. 3 or 6). We took the latter approach because it is slightly more efficient computationally.

Results

For gummy shark, solution of eq. 13 for $\Lambda(a)=\Lambda=-(\lambda+F+M)/\alpha=$ constant by use of Mathematica (Wolfram 1991) yielded $\Lambda(a)=-0.312957\cdot\text{year}^{-1}$. From eq. 3 or 6, $\lambda=-\alpha\Lambda(a)-F-M=0.312957-F-0.1970=0.115957-F$. These calculations apply before and after the inception of the fishery. If $F=0$, or before the inception of the fishery, $\lambda=0.115957\cdot\text{year}^{-1}$.

For school shark, solution of eq. 13 for $\Lambda(a)=\Lambda=-(\lambda+F+M)/\alpha=$ constant by use of Mathematica (Wolfram 1991) yielded $\Lambda(a)=-0.216980\cdot\text{year}^{-1}$. From eq. 3 or 6, $\lambda=-\alpha\Lambda(a)-F-M=0.216980-F-0.1075=0.109480-F$. Again, these calculations apply before and after the inception of the fishery. If $F=0$, or before the inception of the fishery, $\lambda=0.109480\cdot\text{year}^{-1}$.

Thus, although gummy shark has a $100\times\left(\frac{0.312957}{0.216980}-1\right)\%\approx 44.23\%$ higher gross productivity than school shark, its net productivity (its part of gross productivity that is actually used for an increase in number of its population size) is similar to that of school shark because of its higher instantaneous rate of natural mortality. This difference is reflected in differences in their stable population age distributions (Fig. 1), with the mean and variance of ages (from birth at age a_0) of all individuals in the population being given, respectively, by $\mu(a-a_0)=1/0.312957\approx 3.1953$ years and $\sigma^2(a-a_0)=\frac{1}{(-0.312957)^2}\approx 10.2101\ \text{year}^2$ for gummy shark and by $\mu(a-a_0)=1/0.216980\approx 4.6087$ years and $\sigma^2(a-a_0)=\frac{1}{(-0.216980)^2}\approx 21.2403\ \text{year}^2$ for school shark.

Discussion

In this work, we have established an explicit relationship between the intrinsic rate of increase with time $\lambda(a,t)$ of a fish population and its instantaneous rate of natural mortality $M(a,t)$ from an age-dependent population dynamics model, generalized Lotka's (1922) equation for calculating the intrinsic rate of increase with time $\lambda(a,t)$, derived its dual equation for calculating the intrinsic rate of decrease with

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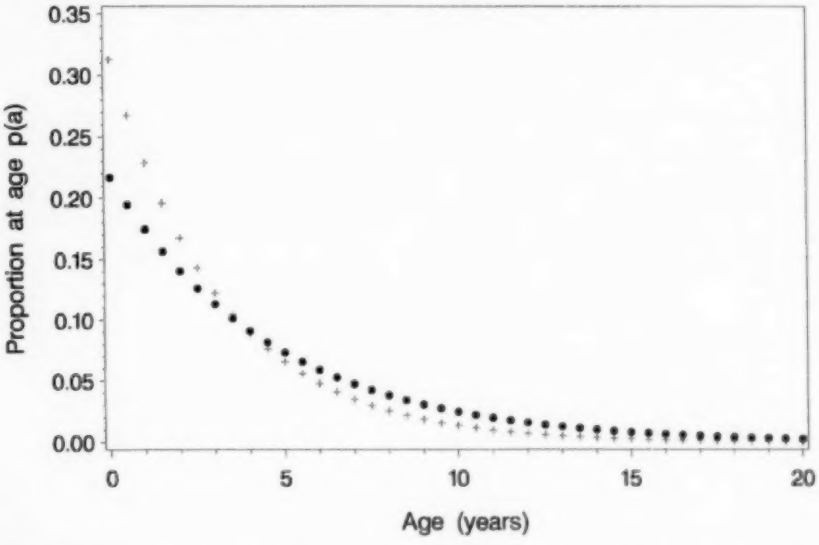
Fig. 1. Stable age distribution $p(a)$ as a function of age a for female gummy shark (+) and female school shark (●) populations off southern Australia.



age $\Lambda(a,t)$, and applied that relationship and both equations to data on gummy shark in Bass Strait and school shark off southern Australia.

Which set of equations, the generalized Lotka (1922) equation (eq. 10 or 11) and its dual equation (eq. 12 or 13) or the generalized Lotka (1922) equation (eq. 19 or 20) and its dual equation (eq. 17 or 18), should be used in calculating the intrinsic rate of increase with time $\lambda(a,t)$ and the intrinsic rate of decrease with age $\Lambda(a,t)$? The answer lies in their assumptions. Equations 10–13 assume that the instantaneous rate of total mortality $Z(a,t) = Z(a)$ is a function of age a only, whereas eqs. 17–20 assume that $Z(a,t) = Z(t)$ is a function of time t only. For unexploited populations, eqs. 10–13 would probably fare better than eqs. 17–20 because $Z(a,t) = Z(a) = M(a)$ would be a reasonable assumption. For exploited populations and if $Z(a,t)$ varies strongly with time t and age a, neither of these assumptions would be met. However, if the instantaneous rate of natural mortality $M(a,t)$ is a constant, eqs. 17–20 would fare better than eqs. 10–13 because the instantaneous rate of fishing mortality $F(a,t)$ is mainly a function of time t. Both sets of equations are identical for constant instantaneous rate of natural mortality $M(a,t)$, constant instantaneous rate of fishing mortality $F(a,t)$, constant intrinsic rate of increase with time $\lambda(a,t)$, and constant intrinsic rate of decrease with age $\Lambda(a,t)$.

It is not surprising that an explicit relationship between the intrinsic rate of increase of a fish population $\lambda(a,t)$ and its instantaneous rate of natural mortality $M(a,t)$ has never been derived before. Population dynamics models, particularly those in fisheries science, have followed a typical, logical progression in their development from age-dependent models to age- and time-dependent models. Their representation as a partial differential equation with respect to both age and time, as is essential for its derivation, has appeared in fisheries literature only rather recently (e.g., Xiao 1997). Both relative rates of change in $N(a,t)$ must then be interpreted properly to obtain that relationship.

This work has major implications for calculating, by use of interspecific models, the instantaneous rate of natural mortality of a fish population $M(a,t)$ from its surrogates, such as the intrinsic rate of increase with time $\lambda(a,t)$. Because of difficulties in directly calculating $M(a,t)$, several interspecific models have been developed for this purpose (e.g., Ohsumi 1979; Gunderson 1980; Pauly 1980; Xiao 1999). However, eq. 3, rewritten as $M(a,t) = -\lambda(a,t) - F(a,t) - \alpha\Lambda(a,t)$, implies that, for a given species, $M(a,t)$ decreases linearly with $\lambda(a,t)$ with a slope of -1 and an intercept of $-F(a,t) - \alpha\Lambda(a,t)$ and that any of its quantities is species specific, including the intercept. If a straight line is fitted to data on $M(a,t)$ and $\lambda(a,t)$ from many species, as in an interspecific model, the intercept is then assumed, implicitly, to be common to all species. This assumption is generally invalid because both gross productivity $-\alpha\Lambda(a,t)$ and fishing mortality $F(a,t)$ differ among species. The same argument applies to other interspecifc models. Therefore, interspecific models are of limited value in calculating the instantaneous rate of natural mortality of a fish population. However, this does not mean that data on $M(a,t)$ and $\lambda(a,t)$ from many species are not useful for other purposes. Indeed, they are potentially very useful for delineating possible intraspecific and interspecific groupings or associations by use of techniques for classification and ordination (Pielou 1984).

Similarly, eq. 3 is sufficient but not necessary for calculating, for a particular species, $M(a,t)$ from $\lambda(a,t)$ or vice versa, so that alternative approaches are possible. Data in Au and Smith (1997) on $M(a,t)$ and $\lambda(a,t)$ will be useful for testing those alternatives. However, for a given species, any alternative must satisfy and hence be constrained by eq. 3. Also, $M(a,t)$ is affected by all other quantities in the generalized Lotka (1922) equation and its dual equation. Relating it to a single quantity, such as the maximum age T, is overly simplistic.

The sex ratio of females at birth and their age at first maturity in a fish population of two sexes must be specified

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Fig. 2. Intrinsic rate of increase with time $\lambda(a,t)$ of female gummy shark (+) and female school shark (●) (when the instantaneous rate of fishing mortality $F(a,t) = 0$) and the intrinsic rate of decrease with age $\Lambda(a,t)$ of female gummy shark (*) and female school shark (○) off southern Australia as a function of the average proportion of the progeny that are female produced by all female fish of age a at time t, $R(a,t)$.

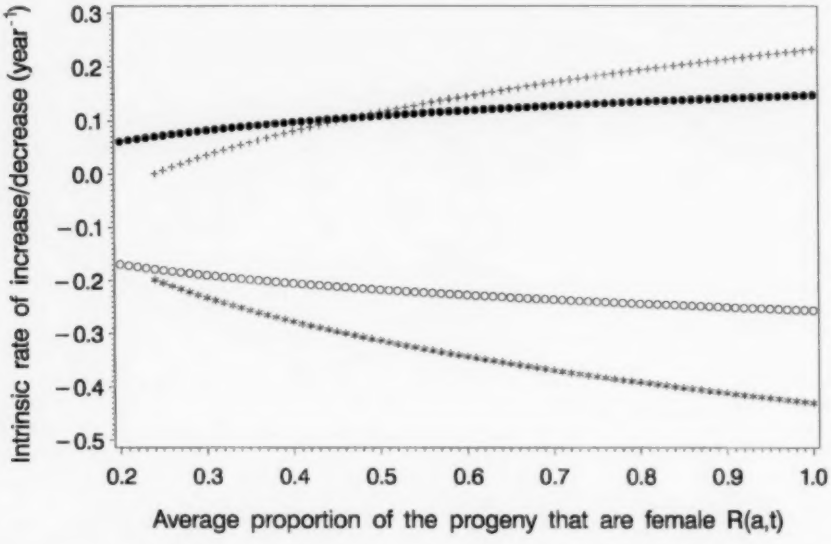
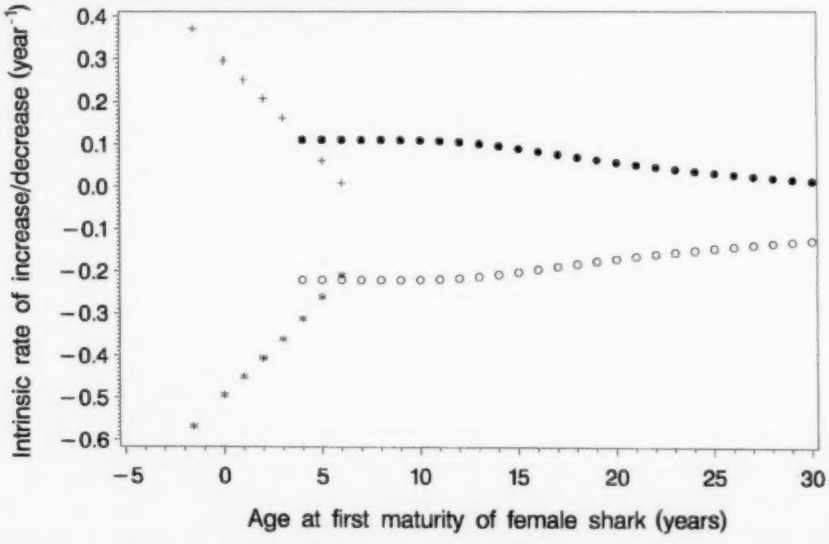


Fig. 3. Intrinsic rate of increase with time $\lambda(a,t)$ of female gummy shark (+) and female school shark (●) (when the instantaneous rate of fishing mortality $F(a,t) = 0$) and the intrinsic rate of decrease with age $\Lambda(a,t)$ of female gummy shark (*) and female school shark (○) off southern Australia as a function of their age at first maturity a_m.



correctly to avoid great uncertainty in calculations of $\lambda(a,t)$ and $\Lambda(a,t)$. For example, for a sex ratio of 1:1 in a fish population of two sexes, $\frac{1}{R} = 2$. However, if $\frac{1}{R} = 1$ is assumed in calculating $\lambda(a,t) = \lambda$ by use of eq. 10 or 11 and $\Lambda(a,t) = \Lambda$ by use of eq. 12 or 13, λ will increase from $0.115957 - F$ to $0.232085 - F \cdot \text{year}^{-1}$ and Λ will decrease from -0.312957 to $-0.429085 \cdot \text{year}^{-1}$ for gummy shark and λ will increase from $0.109480 - F$ to $0.148430 - F \cdot \text{year}^{-1}$ and Λ will decrease from -0.216980 to $-0.255930 \cdot \text{year}^{-1}$ for school shark (Fig. 2). Similarly, λ calculated by use of eq. 10 or 11 will decrease and Λ calculated by use of eq. 12 or 13 will increase with age at first maturity, as in the case of gummy and school shark (Fig. 3). Great care should be taken in specifying the sex ratio of females at birth and their age at first maturity in a fish population of two sexes.

Finally, it is a remarkable finding that the intrinsic rate of increase with time λ of a population of animals is determined by its reproductive schedules $R(a,t)$ and $\beta(a,t)$ and by its instantaneous rate of total mortality $Z(a,t)$ (eq. 10 or 11),

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whereas the intrinsic rate of decrease with age Λ is determined only by its reproductive schedules $R(a,t)$ and $\beta(a,t)$ (eq. 12 or 13). Indeed, as mentioned earlier, eq. 3 implies that $-\alpha\Lambda(a,t)$ is the instantaneous rate of gross productivity of fish of age a at time t in a fish population, $\lambda(a,t)$ is the instantaneous rate of net productivity of fish of age a at time t, and $Z(a,t)$ is the instantaneous rate of productivity of fish of age a at time t lost due to total mortality. Therefore, the intrinsic rate of increase with time $\lambda(a,t)$ of a fish population is a measure of its net reproductive potential and its net resilience to human exploitation, whereas the intrinsic rate of decrease with age times a constant $-\alpha\Lambda(a,t)$ ($\alpha = 1$ if both age a and time t have the same units, e.g., years) is a measure of its gross reproductive potential and its gross resilience to human exploitation.

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References

Au, D.W., and Smith, S.E. 1997. A demographic method with population density compensation for estimating productivity and yield per recruit. Can. J. Fish. Aquat. Sci. **54**: 415–420.

Cailliet, G.M. 1992. Demography of the central California population of the leopard shark (*Triakis semifasciata*). Aust. J. Mar. Freshwater Res. **43**: 183–193.

Cailliet, G.M., Mollet, H.F., Pittenger, G.G., Bedford, D., and Natanson, L.J. 1992. Growth and demography of the Pacific angel shark (*Squatina californica*), based upon tag returns off California. Aust. J. Mar. Freshwater Res. **43**: 1313–1330.

Cortés, E. 1995. Demographic-analysis of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, in the Gulf of Mexico. Fish. Bull. U.S. **93**: 57–66.

Cortés, E. 1998. Demographic analysis as an aid in shark stock assessment and management. Fish. Res. **39**: 199–208.

Cortés, E. 1999. A stochastic stage-based population model of the sandbar shark in the western North Altantic. Trans. Am. Fish. Soc. In press.

Cortés, E., and Parsons, G.R. 1996. Comparative demography of two populations of the bonnethead shark (*Sphyrna tiburo*). Can. J. Fish. Aquat. Sci. **53**: 709–718.

Grant, C.J., Sandland, R.L., and Olsen, A.M. 1979. Estimation of growth, mortality and yield per recruit of the Australian school shark, *Galeorhinus australis* (Macleay), from tag recoveries. Aust. J. Mar. Freshwater Res. **30**: 625–637.

Gunderson, D.R. 1980. Using *r-K* selection theory to predict natural mortality. Can. J. Fish. Aquat. Sci. **37**: 2266–2271.

Hoenig, J.M., and Gruber, S.H. 1990. Life-history patterns in the elasmobranchs: implications for fisheries management. *In* Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries. *Edited by* H.L. Pratt, Jr., S.H. Gruber, and T. Taniuchi. NOAA Tech. Rep. NMFS No. 90. pp. 1–16.

Hoff, T.B., and Musick, J.A. 1990. Western North Atlantic shark-fishery management problems and informational requirements. *In* Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries. *Edited by* H.L. Pratt, Jr., S.H. Gruber, and T. Taniuchi. NOAA Tech. Rep. NMFS No. 90. pp. 455–472.

Hoppensteadt, F. 1975. Mathematical theories of populations: demographics, genetics and epidemics. Society for Industrial and Applied Mathematics, Philadelphia, PA 19103, U.S.A.

Jones, B.C., and Geen, G.H. 1977. Reproduction and embryonic development of spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. **34**: 1286–1292.

Kermack, W.O., and McKendrick, A.G. 1927. Contributions to the mathematical theory of epidemics. II. The problem of endemicity. Proc. R. Soc. Lond. Ser. A, Math. Phys. Sci. **115**: 55–83.

Kermack, W.O., and McKendrick, A.G. 1932. Contributions to the mathematical theory of epidemics. III. Further studies of the problem of endemicity. Proc. R. Soc. Lond. Ser. A, Math. Phys. Sci. **138**: 94–122.

Last, P.R., and Stevens, J.D. 1994. Sharks and rays of Australia. CSIRO Australia.

Lotka, A.J. 1922. The stability of the normal age distribution. Proc. Nat. Acad. Sci. U.S.A. **8**: 339–345.

Lotka, A.J. 1924. Elements of physical biology. Reprinted in 1956 as "Elements of mathematical biology." Dover Publications, Inc., New York.

Márquez-Farias, J.F., and Castillo-Géniz, J.L. 1998. Fishery biology and demography of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, in the southern Gulf of Mexico. Fish. Res. **39**: 183–198.

Márquez-Farias, J.F., Castillo-Géniz, J.L., and Rodríguez de la Cruz, M.C. 1998. Demography of the bonnethead shark, *Sphyrna tiburo* (Linneaus, 1758), in the southeastern Gulf of Mexico. Cienc. Mar. **24**: 13–34.

McKendrick, A.G. 1926. Applications of mathematics to medical problems. Proc. Edinb. Math. Soc. **44**: 98–130.

Moulton, P.M., Walker, T.I., and Saddlier, S.R. 1992. Age and growth studies of gummy shark, *Mustelus antarcticus* Günther, and school shark, *Galeorhinus galeus* (Linnaeus), from southern-Australian waters. Aust. J. Mar. Freshwater Res. **43**: 1241–1267.

Ohsumi, S. 1979. Interspecies relationships among some biological parameters in cetaceans and estimation of the natural mortality coefficient of the Southern Hemisphere minke whale. Rep. Int. Whaling Comm. **29**: 397–406.

Pauly, D. 1980. On the relationship between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. Cons. Int. Explor. Mer. **3**: 175–192.

Pielou, E.C. 1984. The interpretation of ecological data: a primer on classification and ordination. John Wiley & Sons, Chichester, U.K.

Roff, D.A. 1981. Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? Can. J. Fish. Aquat. Sci. **38**: 968–977.

Smirkey, T.R., and Musick, J.A. 1996. Demographic analysis of the sandbar shark, *Carcharhinus plumbeus*, on the western North Atlantic. Fish. Bull. U.S. **94**: 341–347.

Smith, S.E., Au, D.W., and Show, C. 1998. Intrinsic rebound potentials of 26 species of Pacific sharks. Mar. Freshwater Res. **49**: 663–678.

Walker, T.I. 1992. A fishery simulation model for sharks applied to the gummy shark, *Mustelus antarcticus* Günther, from southern Australian waters. Aust. J. Mar. Freshwater Res. **43**: 195–212.

Walker, T.I. 1994. Fishery model of gummy shark, *Mustelus antarcticus*, for Bass Strait. *In* New opportunities: best practice. *Edited by* I. Bishop. Resource Technology '94 Conference Proceedings, Melbourne, 26–30 September 1994. Centre for Geographic Information Systems and Modelling, University of Melbourne, Melbourne, Australia. pp. 422–438.

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Walker, T.I. 1998. Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. Mar. Freshwater Res. **49**: 553–572.

Walker, T.I., Stone, T., Brown, D., and McLoughlin, K. 1997. Fisheries assessment report: the southern shark fishery 1996. Australian Fisheries Management Authority, Canberra, Australia.

Wolfram, S. 1991. Mathematica: a system for doing mathematics by computer. 2nd ed. Addison-Wesley Publishing Company, Inc., Reading, Mass.

Woodley, T.H., and Read, A.J. 1991. Potential rates of increase of a harbour porpoise (*Phocoena phocoena*) population subjected to incidental mortality in commercial fisheries. Can. J. Fish. Aquat. Sci. **48**: 2429–2435.

Xiao, Y. 1997. Subtleties in, and practical problems with, the use of production models in fish stock assessment. Fish. Res. **33**: 17–36.

Xiao, Y. 1999. Formulae for calculating the instantaneous rate of natural mortality of animals from its surrogates. Math. Comput. Model. In press.

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Density-dependent habitat selection and the modeling of sperm whale (*Physeter macrocephalus*) exploitation

Hal Whitehead

Abstract: The monitoring and management of sperm whale (*Physeter macrocephalus*) populations have proved problematic. Studies of living animals indicate that movements are largely determined by resource availability, thus suggesting that density-dependent habitat selection may be a realistic framework within which to study sperm whale populations. A model, in which animals migrate between 2 × 2° squares at rates that depend on relative resource availability, was used to examine the effects of whaling on measures of sperm whale abundance. The model simulated four types of whaling: shore-based whaling, pelagic open-boat whaling by many boats, pelagic whaling by a fleet based around one factory ship, and pelagic whaling by a fleet sequentially exploiting different parts of the study area. Catch per unit effort was found to have little relationship with population size in any part of the study area for shore-based whaling and for pelagic whaling when the study area was sequentially exploited. Thus, in these circumstances, catch per unit effort should not be used as a measure of depletion. To give a reasonable assessment of depletion, visual or acoustic surveys must extend well beyond the areas being exploited.

Résumé : La surveillance et la gestion des populations de cachalots macrocéphales (*Physeter macrocephalus*) se sont avérées problématiques. Les études sur les animaux vivants montrent que leurs déplacements sont largement déterminés par les ressources disponibles, ce qui laisse penser qu'il serait réaliste d'étudier les populations de cachalots macrocéphales en fonction d'une sélection de l'habitat dépendante de la densité. On a utilisé un modèle dans lequel les animaux migrent entre des carrés de 2° × 2° à des rythmes qui dépendent des ressources disponibles pour examiner les effets de la chasse sur les mesures de l'abondance des cachalots macrocéphales. Le modèle simulait quatre types de chasse : chasse depuis le rivage, chasse pélagique réalisée par de nombreux bateaux non pontés, chasse pélagique réalisée par une flottille rattachée à un bateau-usine, et chasse pélagique réalisée par une flottille exploitant séquentiellement différentes zones de l'aire de l'étude. On a observé que les captures par unité d'effort étaient peu reliées à la taille de la population partout dans l'aire de l'étude dans les cas de la chasse depuis le rivage et de la chasse pélagique séquentielle. Ainsi, dans ces conditions, les captures par unité d'effort ne devraient pas être utilisées comme mesure de la déplétion des populations. Pour obtenir une évaluation raisonnable de cette déplétion, on devrait effectuer des relevés visuels ou acoustiques s'étendant bien au-delà des zones exploitées.

[Traduit par la Rédaction]

Introduction

During the 1970s and early 1980s the Scientific Committee of the International Whaling Commission made considerable efforts to model sperm whale (*Physeter macrocephalus*) populations with the intention of better managing exploitation (e.g., International Whaling Commission 1980). The application of the methods that were developed have problems for a number of reasons, which include uncertainties about natural history parameters (Best et al. 1984), misreporting of data (Zemsky et al. 1995), the effects of adult sex ratio and social structure (Botkin et al. 1980), as well as fundamental issues concerned with assessment mechanisms (Cooke 1986).

One of the most controversial and difficult issues that the Scientific Committee of the International Whaling Commission faced during these assessments concerned stock boundaries (Donovan 1991). There is evidence from artificial and natural marking studies that, while some sperm whales may be resighted within a small area after intervals of months or years, others can move 1000 km or more (Kasuya and Miyashita 1988; Dufault and Whitehead 1995). A review of studies of sperm whale stock structure, including recent genetic work, suggested that there are no clear stock divisions, defined by lines that animals rarely cross, for sperm whales in the world's oceans (Dufault et al. 1999). Thus, a model of sperm whale populations based on discrete stocks is likely to be fundamentally invalid.

Recent research has examined the movement patterns of living animals. It seems that the movements of groups of female sperm whales are related to feeding success. When feeding success is high, groups zigzag back and forth over areas a few tens of kilometres across, whereas when success is low, they move in straight lines at speeds of about 100 km/day (Whitehead 1996; Jaquet and Whitehead 1999). This means that, within areas a few tens or hundreds of kilometres across, residence times are long when feeding success is high and short when it is low (Whitehead 1996).

Such movement patterns can lead to a system of density-

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dependent habitat selection in which animals tend to distribute themselves geographically so that feeding success is roughly equal everywhere (MacCall 1990). Whitehead et al. (1997) have suggested that density-dependent habitat selection may explain a decline in the number of sperm whales off the Galápagos as animals redistributed themselves following the end of intense whaling off Peru.

In this paper, I explore how density-dependent habitat selection and whaling might interact for sperm whales and the implications for the management of populations. I use a simulation model in which whales move between ocean areas depending on relative resource availability and then allow the population to be exploited in a variety of ways (as suggested by Hilborn and Walters (1987) and MacCall (1990)). I then examine how catch per unit effort (CPUE) is related to population size. The model used is simplistic, but parameters are chosen to approximate reality.

Methods

Model of density-dependent habitat selection for sperm whales

A simple model of density-dependent habitat selection of sperm whales was constructed (using Matlab 5.2) with the following characteristics.

(*i*) Two-dimensional array of squares indexed by i ($i = 1,..., I$) (a one-dimensional index is used to simplify the notation). Each has a population of whales, n_i, that varies with time, and a resource availability, r_i, that, in some versions, varies with time.

(*ii*) Movement of whales between squares. Each square has at most four surrounding squares. Transition probabilities from square i to square j at the start of each time unit, $P(i,j)$, are given by

$$P(i,j) = abn_i r_j^2/[(r_i^2 + bn_i)(r_j^2 + bn_j)] \text{ if } i \text{ and } j \text{ are neighbouring squares}$$

$$P(i,j) = 0 \text{ if } i \text{ and } j \text{ are not neighbouring squares}$$

$$P(i,j) = 1 - \sum_{j \neq i} P(i, j),$$

where a and b are constants with $0 < a \leq 0.25$ and $b > 0$. Constant a can be interpreted as the overall rate of migration during a time period, and constant b as expressing the relative importance of resource abundance per animal in the source (high b) and receiving squares (low b) in determining migration rates. Constant b should have a value comparable with the n values. This expression was chosen as it has the following properties (when i and j are neighbouring squares): $P(i,j) \geq 0$; $n_i P(i,j)$ increases as r_i/n_i decreases: more emigration with less resource per animal in source square; $n_i P(i,j)$ decreases as r_j/n_j decreases: less emigration with less resource per animal in receiving square; $\Sigma P(i,j) \leq 1$(with summation over all neighbouring squares j for each i): total emigration from a square is less than the number of animals in it; and $n_i P(i,j) = n_j P(j,i)$ when $r_i/n_i = r_j/n_j$: no net migration between neighbouring squares when resource per animal is equal in them. Although there are other expressions that satisfy these conditions, they are restrictive. This migration model is illustrated in Fig. 1.

(*iii*) Whaling. Animals are then removed from square i as follows in each time unit, so that

$$n_i(t + 1) = [\sum_{j=1,...,I} n_j(t) P(j,i)] s_i(t + 1),$$

where $s_i(t)$ is the selectivity in square i at time t.

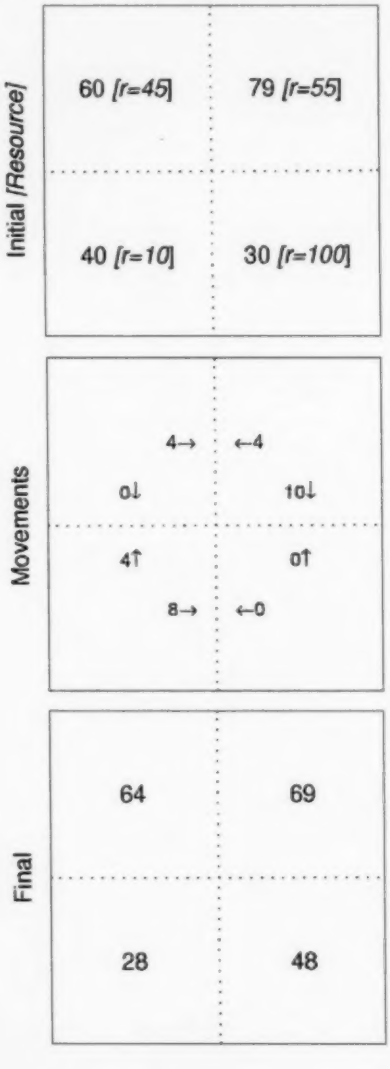

Fig. 1. Illustration of the density-dependent movement model. Initial whale numbers and resource availabilities over four adjacent squares illustrated in the upper diagram trigger movements of whales between squares (centre diagram), and thus a distribution of whales one time unit later shown in the lower diagram. Note that the proportion of whales moving to an adjacent square depends on the relative resource availabilities, per whale, in the two squares.

Parameterization of model

The form and parameters of the model were chosen to roughly approximate what is known about the biology of the female and immature sperm whales in the eastern tropical Pacific Ocean. In this area, there is little evidence for seasonal or breeding migrations by females and immatures (e.g., Hope and Whitehead 1991). No attempt has been made to incorporate the migrations of adult males into, and out of, the study area, as these animals constitute only a few percent of the population (Hope and Whitehead 1991) and little is known of their migrations.

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Fig. 2. Initial distribution of resource availability, and sperm whales, in 2° squares of the eastern tropical Pacific from the charts of Townsend (1935). Darker shading indicates more resources and more animals. The white squares on the eastern side of the map represent the South and Central American mainland and are not considered in the simulations.

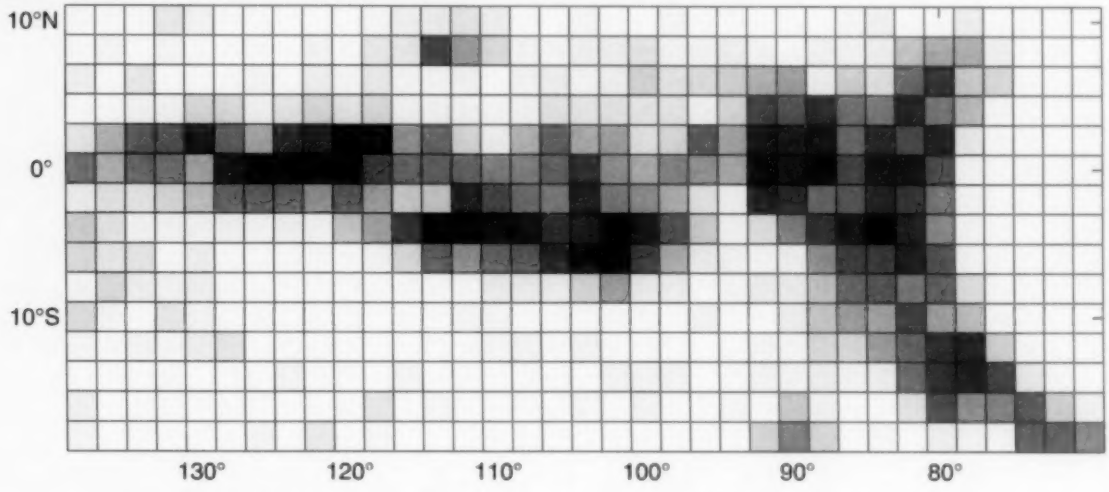

Table 1. Estimated initial population sizes from the *x*-intercept of the linear regression of CPUE on cumulative catch during the first and second decades of whaling for four types of whaling and two implementations of resource availability (results for two runs with temporally variable resources are separated by a solidus).

	Resource availability			
	Temporally constant		Temporally variable	
Type of whaling	First decade	Second decade	First decade	Second decade
Shore based	17 730	25 501	15 101/14 470	22 938/23 061
Pelagic, open boat	49 950	49 950	49 950/49 950	49 950/49 950
Pelagic, factory fleet	38 539	43 932	40 443/39 801	43 808/43 352
Pelagic, sequential exploitation	42 548	–287 758	50 035/53 801	–36 032/–16 635 657

Note: The true initial population size was 50 000 animals.

Time units were weeks, and the simulations were run over 19.2 years (1000 time units). The study area is the eastern tropical Pacific bounded by 20°S, 11°N, 79°W, and 140°W. The study area was divided into 2° latitude × 2° longitude squares. Land areas within the study area were not considered, leaving 472 squares.

The initial resource availability in a square, $r_i(0)$, was proportional to the number of plots of sperm whale kills in that square in the maps of Townsend (1935), as tabulated by Jaquet et al. (1996) plus 0.5. The total resource availability over the study area was 50 000. Two implementations were considered: (*i*) temporally constant resource availability and (*ii*) temporally variable resource availability, with some spatial and temporal autocorrelation, so that a measure of the relative resource availability in square i at time t is given by

$$q_i(0) = 0.1z_i(0)$$

$$q_i(t) = 0.95[6q_i(t-1) + \Sigma q_j(t-1)]/(6 + m_i) + 0.05z_i(t)$$

where the summation is over all neighbouring squares j, m_i is the number of neighbouring squares of square i, and z is a normal random variable with mean 0 and variance 1. From these q_is, the absolute resource availability in square i at time t was calculated from

$$r_i(t) = r_i(0)\exp[12q_i(t)].$$

This produces $r_i(t)$ with a geometric mean equal to $r_i(0)$. The parameters and form of this model were chosen so that the mean CV for each square (0.95) and mean autocorrelation between adjacent weeks (0.57), respectively, equaled the estimated CV and autocorrelation between weekly defecation rates, a measure of feeding success, measured from sperm whales off the Galápagos Islands (data from Whitehead 1996). As in the data on defecation rates, the resource trajectories produced by this model showed increasing variance over time scales up to about 6 months.

The initial populations in each square ($n_i(0)$) equaled the initial resource availability ($r_i(0)$), as indicated in Fig. 2, and both are based on the distribution of sperm whale kills plotted by Townsend (1935). Thus, the total initial population of sperm whales in the area equaled 50 000 animals. This follows the suggestion that the density of plots on Townsend's (1935) charts likely correlates well with prewhaling sperm whale distributions (Whitehead and Jaquet 1996) and is roughly consistent with Wade and Gerrodette's (1993) census estimate of 22 700 sperm whales in the eastern tropical Pacific, given that they made no correction for whales being underwater and so invisible during their surveys.

In the movement model, parameter a was set at 0.25, indicating that for nonborder squares (with four surrounding squares) in extreme cases with $n_i >> r_i$ and $n_j << r_j$, virtually all animals in a

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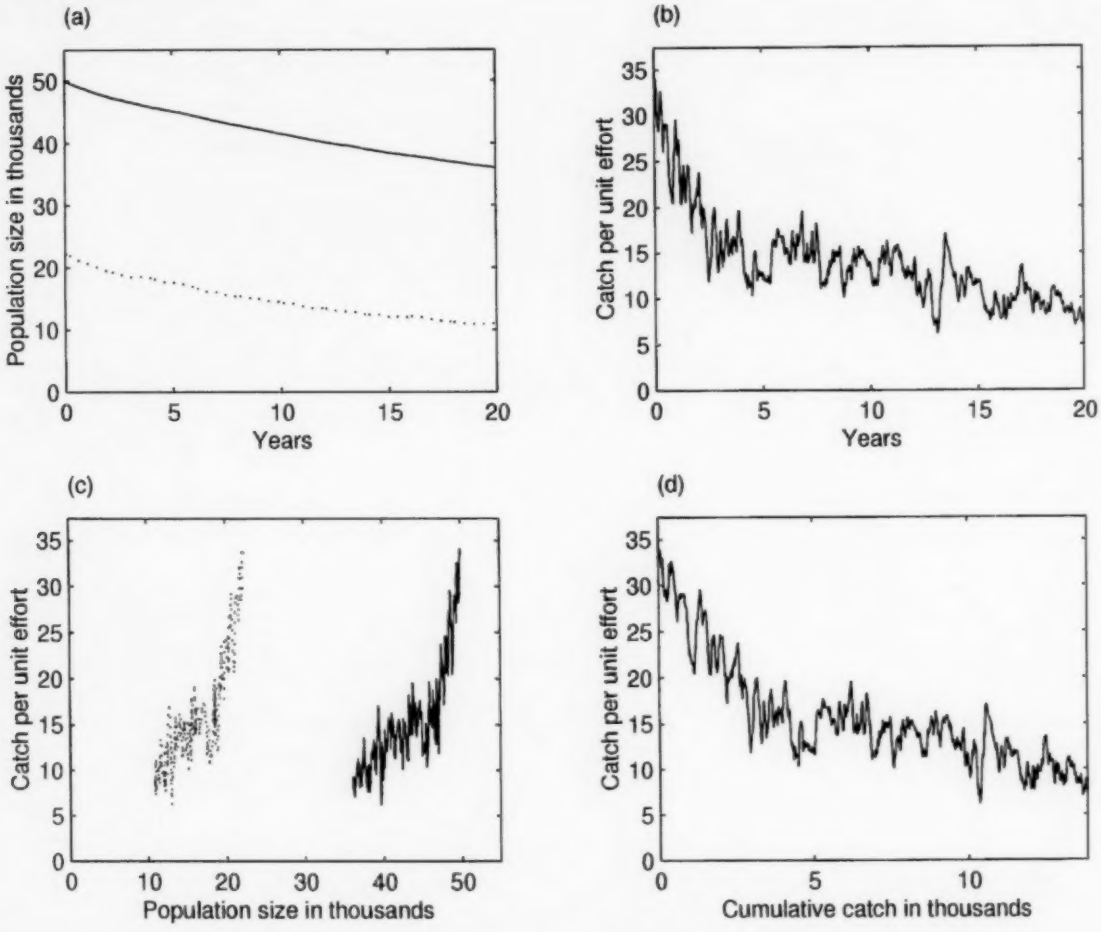
Fig. 3. Results of a model of sperm whale density-dependent habitat selection with shore-based whaling showing (*a*) population size (solid line for the entire study area; dottted line for the area east of 95°W) against time and CPUE against (*b*) time, (*c*) population size, and (*d*) cumulative catch.



square will leave during 1 week. Parameter b was set to the mean resource availability over the study area (105.9 = 50 000/472). In the case of temporally variable resource availabilities, the mean migration rate out of 2° squares was about 0.12/week, translating into root mean square displacements of about 130 km/week, roughly that calculated from photographic individual identification records of female and immature sperm whales in the eastern tropical Pacific using likelihood methods (H. Whitehead, in preparation).

Four types of whaling were simulated, each assuming a constant level of effort in each week and that, in all squares being whaled, the number of whales removed during any time unit was a constant proportion of the number present at the start of a time unit (i.e., s_i is constant over whaled squares). Effort levels were such that at the end of the 19-year simulation, approximately 40% of the animals remained in the eastern part of the study area (roughly the rate of depletion achieved by nineteenth century whalers in the area; Hope and Whitehead 1991). The types of whaling were as follows.

(1) Shore-based whaling. Whaling takes place within the area bounded by 4–10°S, 78–84°W simulating the whaling from Paita, Peru, during the 1960s and 1970s (Ramirez 1989). Two percent of the whales within this area are killed each week, indicating constant effort (s_i = 0.02 in whaled squares; s_i = 0.0 elsewhere).

(2) Pelagic, open-boat whaling. Whaling takes place throughout the study area with 0.1% of the whales in each square being removed each week. This simulates the operation of a fairly large number of whaling vessels that distribute themselves proportional to whale abundance and may roughly approximate whaling by open-boat whalers working from sailing vessels during the late eighteenth and early nineteenth centuries (Whitehead and Hope 1991) (s_i = 0.001 in all squares).

(3) Pelagic, factory-fleet whaling, with scouting. Each week, whaling takes place within the one square that has the highest whale abundance. Ten percent of the whales in this square are removed during the week (s_i = 0.1 in whaled square; s_i = 0.0 elsewhere).

(4) Pelagic, factory-fleet whaling exploiting sequential sectors. This is similar to type 3, except that during each 4-year period, whaling takes place exclusively in a longitudinal swath 12° wide. The first swath is at the western end of the study area, and, as time progresses, whaling effort moves steadily from west to east. This mimics the progress of pelagic sperm whaling in the North Pacific between 1954 and 1976 (Ohsumi 1980).

Output

For each simulation, the program outputs trajectories of the total

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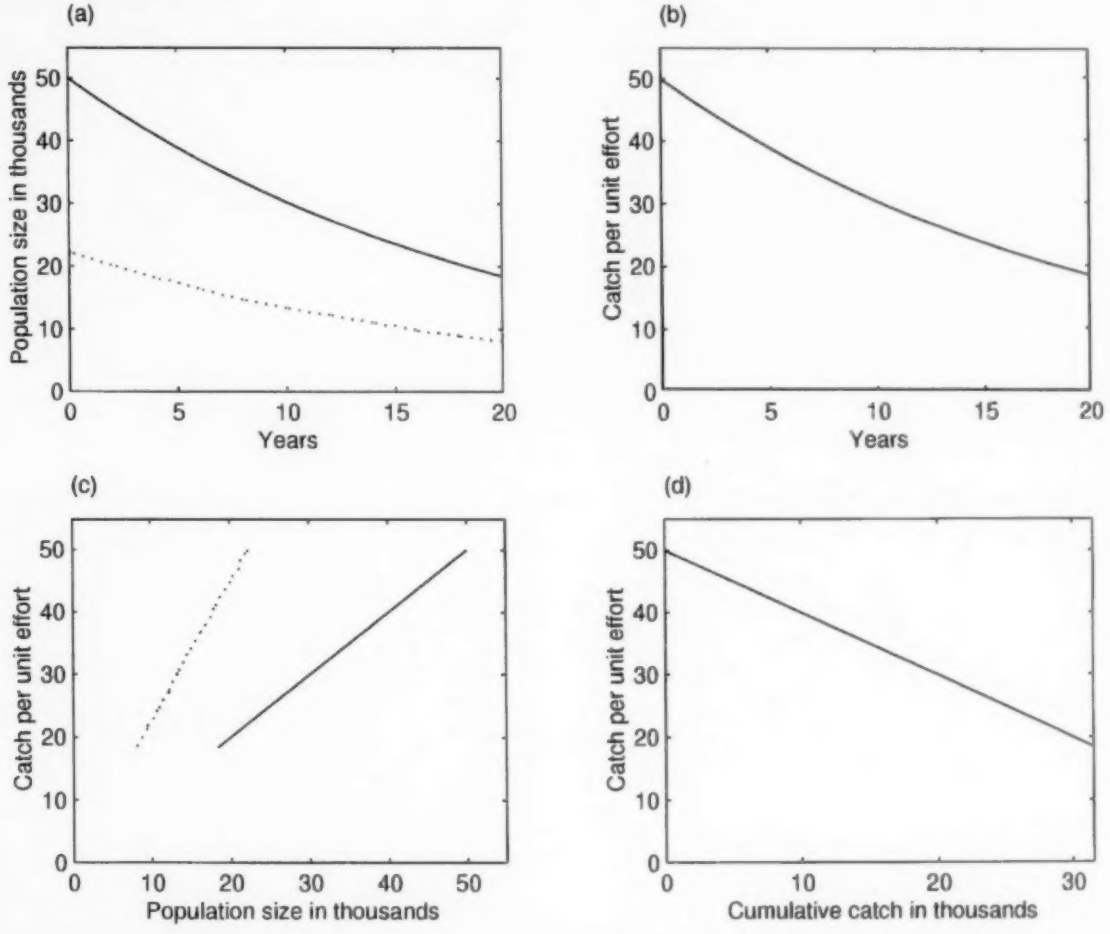
Fig. 4. Results of a model of sperm whale density-dependent habitat selection with pelagic, open-boat whaling showing (*a*) population size (solid line for the entire study area; dotted line for the area east of 95°W) against time and CPUE against (*b*) time, (*c*) population size, and (*d*) cumulative catch.



population size, as well as the population size east of 95°W, with time and trajectories of CPUE, which is proportional to catch (as effort is constant over time in all simulations). It also plots CPUE versus total population size, as well as population size east of 95°W, and CPUE versus cumulative catch. The initial population size is estimated from the *x*-intercept of a linear regression of CPUE on cumulative catch (Leslie and Davis 1939) during both the first decade of whaling and the second (to remove special conditions consequent to the initiation of exploitation).

Results

Results are shown in Table 1 and Figs. 3–6. The plots relating catch, population size, and time differed little whether resource availability was constant or variable, although there was greater variability about the trend lines with temporally variable resources. There were also no qualitative differences between replicate runs of the model with temporally variable resources. Thus, only the trajectories for the initial run with variable resource availability are shown in Figs. 3–6.

With shore-based whaling, CPUE starts high, falls quickly as the animals in the whaling area are removed, and then levels out as the whalers catch immigrants from surrounding areas (Fig. 3), showing the "hyperdepletion" pattern of Hilborn and Walters (1992). In this situation, the CPUE trajectory bears little resemblance to changes in population size in the whole study area, the area east of 95°W, the whaling area, or any other area, however defined. The estimate of initial population size from CPUE against cumulative catch regression (Table 1) has little use, as its value depends on what time period is chosen for the regression, and it is not clear for what area the population is being estimated.

With pelagic, open-boat whaling, the situation is very different, with linear relationships between CPUE and population size of the whole area and its eastern portion (Fig. 4), as well as an accurate estimate of initial population size (Table 1).

With pelagic, factory-fleet whaling efficiently exploiting the entire study area, CPUE is monotonically related to population size (Fig. 5), although initial population size is underestimated (Table 1) because CPUE falls faster than population size.

Finally, when the study area is exploited sequentially, CPUE shows no consistent trend with time or population

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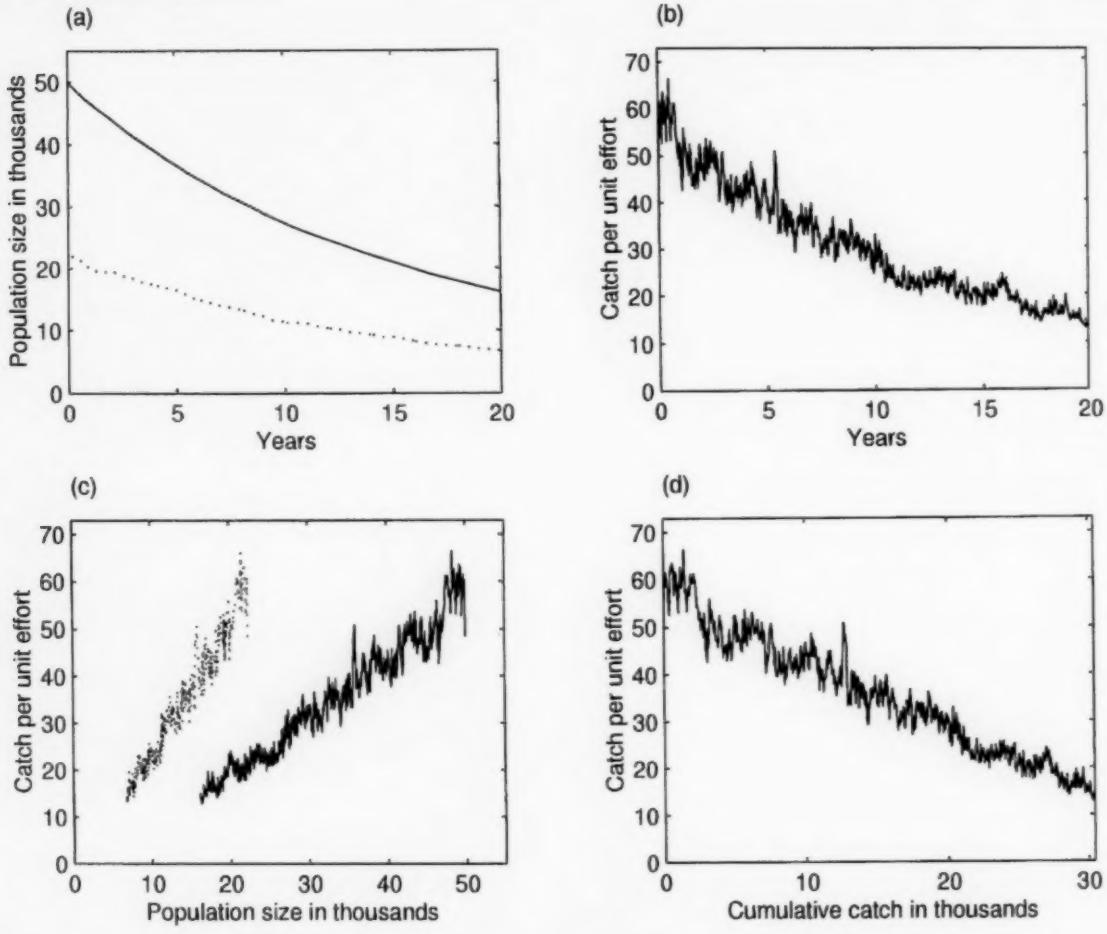
Fig. 5. Results of a model of sperm whale density-dependent habitat selection with pelagic, factory-fleet whaling showing (*a*) population size (solid line for the entire study area; dotted line for the area east of 95°W) against time and CPUE against (*b*) time, (*c*) population size, and (*d*) cumulative catch.



size (Fig. 6), an extreme version of the "hyperstability" pattern of Hilborn and Walters (1992). Estimates of initial population size are very variable, even between replicate runs, and sometimes negative (Table 1), indicating their lack of validity. There are quite different trends in abundance in the eastern and western parts of the study area (Fig. 6).

Discussion

Assessing the status of sperm whale populations

This model, like all others, simplifies reality. It could be made more realistic by adding additional details of the biology of the sperm whales and the actions of the whalers (see Hilborn and Walters (1987) for possible methods and examples). Certain attributes of the animals that have been omitted from the model, such as natural mortality and recruitment, are unlikely to have much impact on the results: sperm whales have very low fecundity and natural mortality (Rice 1989) and these will effectively cancel each other out over the period of the simulation. In contrast, some assumptions of the model, particularly those that relate to movements, critically affect the quantitative outputs of the simulations (as expressed in Table 1 and Figs. 3–6). However (as indicated by some experimentation with input parameters), the general qualitative patterns seem quite robust. In particular, it seems reasonable to draw general conclusions about the likely validity of methods of assessing population depletion during different exploitation regimes.

CPUE

The simulations used in this paper indicate that when effort is concentrated in one part of an ocean to which whales can move from other parts, as in shore-based whaling, or when the focus of effort shifts steadily through an ocean, then CPUE has little use as a measure of the relative depletion of sperm whale stocks. In contrast, the simulations suggest that when pelagic whaling effort is reasonably uniform throughout a large and fairly isolated study area, then CPUE may be a good indicator of depletion. However, this depends on CPUE being linearly related to population density over

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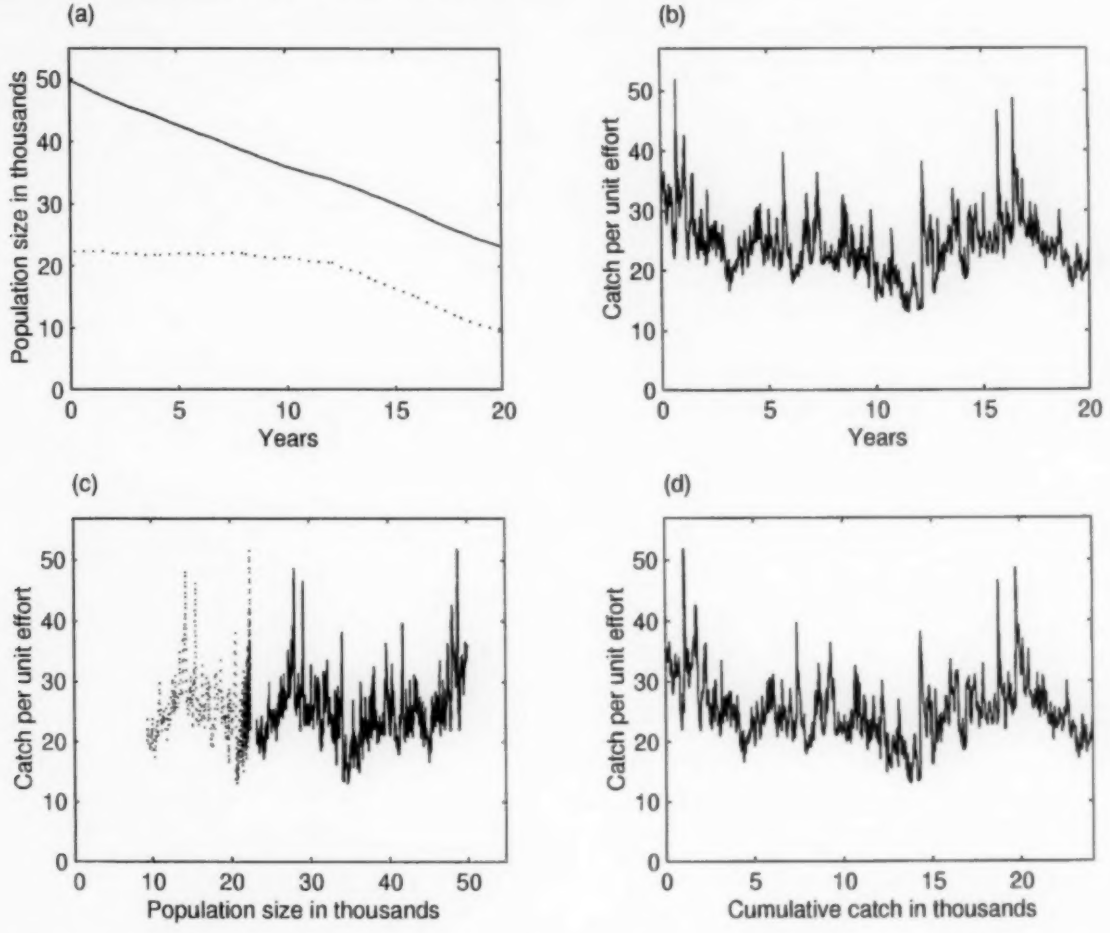
Fig. 6. Results of a model of sperm whale density-dependent habitat selection with pelagic, factory-fleet whaling exploiting sequential areas showing (*a*) population size (solid line for the entire study area; dotted line for the area east of 95°W) against time and CPUE against (*b*) time, (*c*) population size, and (*d*) cumulative catch.



small scales. Cooke (1985) and others have examined this assumption for sperm whales, concluding that it is unlikely to be generally tenable. Thus, even without the questions of spatial dynamics considered in this paper, CPUE is usually not a good measure of sperm whale abundance.

Sightings surveys

Although this modeling does not specifically consider visual or acoustic surveys as techniques for assessing abundance, it is possible to draw some conclusions about their possible efficacy in assessing population status. If we assume that the estimated density of animals from such a survey is proportional to the population size in the survey area during the survey, then very large scale surveys can indicate the status of depletion of the population in an ocean basin. However, surveys of smaller areas may be very misleading. Estimates of density from surveys in the area of whaling operations should be approximately proportional to CPUE. However, as shown in Figs. 3 and 6, this can be very poorly related to the status of the population. In particular, with shore-based whaling or sequential exploitation, regular surveys must extend well beyond the area in which catches are being made and sample distant regions thoroughly, if the true impact of the exploitation is to be assessed.

Even when surveys are intense, wide-ranging, and regular, the possibility of spatial or temporal variability in the relationship between the number of animals present and the density estimated from surveys can cause problems. This could result from variability in the probability of contact in different oceanographic or climatic conditions (e.g., visibility for visual surveys or background noise for acoustic surveys) or changes in whale behaviour (such as the proportion of time spent at the surface or the mean group size) with geographical area, time period, or intensity of exploitation (Cooke 1986; Whitehead 1999).

Length-specific methods

The most recent attempts to examine the effects of whaling on sperm whale populations used temporal changes in length distributions as indicators of depletion (Cooke and de

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la Mare 1983). Although I have not attempted to model length distributions, it is clear that, in the case of density-dependent habitat selection, the length distribution of catches will depend on the geographical extent of whaling and the rates of migration into the whaling area. Thus, without an accurate model of migrations, length-specific methods cannot be expected to give accurate depictions of how geographically restricted whaling operations affect sperm whale populations. Length-specific methods have other problems, summarized by Cooke (1986).

Conclusions

This consideration of density-dependent habitat selection of sperm whales adds further weight to arguments that CPUE data are not useful in assessing depletion levels of this species, except possibly in the case of very widespread geographically unrestricted pelagic whaling, as happened with eighteenth and nineteenth century open-boat whalers. It also shows that if sightings surveys are used to monitor the effects of geographically restricted whaling, then they should be carried out over a much wider area than the exploitation. If we are to monitor the effects of exploitation, or other anthropogenic threats, on sperm whale populations with any reliability, then we need a realistic model of sperm whale movement patterns.

Acknowledgements

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References

Best, P.B., Canham, P.A.S., and Macleod, N. 1984. Patterns of reproduction in sperm whales, *Physeter macrocephalus*. Rep. Int. Whaling Comm. Spec. Issue, **6**: 51–79.

Botkin, D.B., Schimel, D.S., Wu, L.S., and Little, W.S. 1980. Some comments on density dependent factors in sperm whale populations. Rep. Int. Whaling Comm. Spec. Issue, **2**: 83–88.

Cooke, J.G. 1985. On the relationship between catch per unit effort and whale abundance. Rep. Int. Whaling Comm. **35**: 511–519.

Cooke, J.G. 1986. A review of some problems relating to the assessment of sperm whale stocks, with reference to the western North Pacific. Rep. Int. Whaling Comm. **36**: 187–190.

Cooke, J.G., and de la Mare, W.K. 1983. Description of and simulation studies on the length-specific sperm whale assessment technique. Rep. Int. Whaling Comm. **33**: 741–745.

Donovan, G.P. 1991. A review of IWC stock boundaries. Rep. Int. Whaling Comm. Spec. Issue, **13**: 39–68.

Dufault, S., and Whitehead, H. 1995. The geographic stock structure of female and immature sperm whales in the South Pacific. Rep. Int. Whaling Comm. **45**: 401–405.

Dufault, S., Whitehead, H., and Dillon, M. 1999. An examination of the current knowledge on the stock structure of sperm whales (*Physeter macrocephalus*) worldwide. J. Cetacean Res. Manage. **1**: 1–10.

Hilborn, R., and Walters, C.J. 1987. A general simulation model for simulation of stock and fleet dynamics in spatially heterogeneous fisheries. Can. J. Fish. Aquat. Sci. **44**: 1366–1369.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, London, U.K.

Hope, P.L., and Whitehead, H. 1991. Sperm whales off the Galápagos Islands from 1830–50 and comparisons with modern studies. Rep. Int. Whaling Comm. **41**: 273–286.

International Whaling Commission. 1980. Report of the Special Meeting on North Pacific Sperm Whale Assessments, Cronulla, November 1977. Rep. Int. Whaling Comm. Spec. Issue, **2**: 1–10.

Jaquet, N., and Whitehead, H. 1999. Movements, distribution and feeding success of sperm whales in the Pacific Ocean, over scales of days and tens of kilometers. Aquat. Mamm. **25**: 1–13.

Jaquet, N., Whitehead, H., and Lewis, M. 1996. Coherence between 19th century sperm whale distributions and satellite-derived pigments in the tropical Pacific. Mar. Ecol. Prog. Ser. **145**: 1–10.

Kasuya, T., and Miyashita, T. 1988. Distribution of sperm whale stocks in the North Pacific. Sci. Rep. Whales Res. Inst. Tokyo, **39**: 31–75.

Leslie, P.H., and Davis, D.H.S. 1939. An attempt to determine the absolute number of rats on a given area. J. Anim. Ecol. **8**: 94–113.

MacCall, A.D. 1990. Dynamic geography of marine fish populations. Washington Sea Grant, University of Washington Press, Seattle, Wash.

Ohsumi, S. 1980. Catches of sperm whales by modern whaling in the North Pacific. Rep. Int. Whaling Comm. Spec. Issue, **2**: 11–18.

Ramirez, P. 1989. Captura de cachalote en Paita: 1976–1981. Bol. Lima, **63**: 81–88.

Rice, D.W. 1989. Sperm whale. *Physeter macrocephalus* Linnaeus, 1758. *In* Handbook of marine mammals. Vol. 4. *Edited by* S.H. Ridgway and R. Harrison. Academic Press, London, U.K. pp. 177–233.

Townsend, C.H. 1935. The distribution of certain whales as shown by the logbook records of American whaleships. Zoologica, **19**: 1–50.

Wade, P.R., and Gerrodette, T. 1993. Estimates of cetacean abundance and distribution in the eastern tropical Pacific. Rep. Int. Whaling Comm. **43**: 477–493.

Whitehead, H. 1996. Variation in the feeding success of sperm whales: temporal scale, spatial scale and relationship to migrations. J. Anim. Ecol. **65**: 429–438.

Whitehead, H. 1999. Variation in the visually observable behavior of groups of Galápagos sperm whales. Mar. Mammal Sci. **15**: 1181–1197.

Whitehead, H., and Hope, P.L. 1991. Sperm whalers off the Galápagos Islands and in the western North Pacific, 1830–50: ideal free whalers? Ethol. Sociobiol. **12**: 147–161.

Whitehead, H., and Jaquet, N. 1996. Are the charts of Maury and Townsend good indicators of sperm whale distribution and seasonality? Rep. Int. Whaling Comm. **46**: 643–647.

Whitehead, H., Christal, J., and Dufault, S. 1997. Past and distant whaling and the rapid decline of sperm whales off the Galápagos Islands. Conserv. Biol. **11**: 1387–1396.

Zemsky, V.A., Berzin, A.A., Mikhaliev, Y.A., and Tormosov, D.D. 1995. Soviet Antarctic pelagic whaling after WWII: review of actual catch data. Rep. Int. Whaling Comm. **45**: 131–135.

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Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*

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Abstract: Environmental factors associated with the occurrence of toxic cyanobacterial blooms and toxin production were investigated during the summers of 1994 and 1995 in Steilacoom Lake, Washington. A pronounced and prolonged toxic bloom of *Microcystis aeruginosa* occurred during summer 1994 but not during 1995. Lake characteristics that were associated with the toxic bloom in 1994 were higher total phosphorus, decreased water transparency, high water column stability, high surface water temperature and pH, and decreased lake flushing. Decreased water transparency during 1994 may have been due to significantly lower zooplankton abundance. We hypothesize that this decreased transparency was caused by increased planktivory by higher numbers of coho salmon (*Oncorhynchus kisutch*) fingerlings during 1994 and (or) inhibition of zooplankton grazing by *Microcystis*. The success of *Microcystis* over other cyanobacteria was associated with low nitrogen to phosphorus ratios and low nitrate-nitrogen with sufficient ammonium-nitrogen concentrations. Toxin production (i.e., micrograms of microcystin per gram of plankton biomass) was not constant over the duration of detectable toxicity; hence, no relationship was found between *Microcystis* abundance and microcystin concentration. However, microcystin concentration was positively correlated with increasing soluble reactive phosphorus concentrations between 1 and 10 $\mu g \cdot L^{-1}$, indicating that toxin production may have been limited by phosphorus.

Résumé : On a étudié les facteurs environnementaux responsables des proliférations de cyanobactéries toxiques et de la production de toxines durant les étés 1994 et 1995 dans le lac Steilacoom, dans l'état de Washington. Il y a eu prolifération importante et prolongée de la bactérie toxique *Microcystis aeruginosa* à l'été 1994, mais pas en 1995. Les caractéristiques du lac associées à la prolifération de 1994 étaient les suivantes : phosphore total plus élevé, turbidité de l'eau plus élevée, grande stabilité de la colonne d'eau, température et pH des eaux de surface élevés, et vidange du lac moins importante. La moins grande transparence de l'eau en 1994 pourrait avoir été due à l'abondance significativement plus faible de zooplancton. Nous supposons que cette transparence réduite était de fait due à la présence en 1994 d'un plus grand nombre de juvéniles de saumon coho qui auraient consommé davantage de zooplancton et (ou) à l'inhibition par *Microcystis* du broutage effectué par le zooplancton. La dominance de *Microcystis* sur les autres cyanobactéries était associée à de faibles ratios N:P et à des concentrations d'azote sous la forme de nitrate (NO_3^--N) faibles et d'azote sous la forme d'ammonium (NH_4^+-N) suffisantes. La production de toxine (µg microcystine/g biomasse planctonique) n'était pas constante durant la période où la toxicité était détectable; ainsi, on n'a pu établir de relation entre l'abondance de *Microcystis* et la concentration de microcystine. Cependant, cette dernière était corrélée positivement avec les concentrations de phosphore réactif soluble entre 1 et 10 $\mu g \cdot L^{-1}$, ce qui indique que la production de toxines pourrait avoir été limitée par le phosphore.

[Traduit par la Rédaction]

Introduction

Mass occurrences or "blooms" of cyanobacteria (also referred to as blue-green algae) occur in freshwater and estuarine ecosystems throughout the world. Cyanobacterial blooms are often a manifestation of accelerating eutrophication (i.e., nutrient, particularly phosphorus, enrichment) of aquatic systems. Cyanobacterial blooms may cause a variety of water quality problems including dissolved oxygen (DO) depletion and subsequent fish kills, aesthetic nuisances (e.g., odors, scums, fish tainting, unsightliness), and unpalatable and possibly unsafe drinking water (e.g., Paerl 1988; Welch 1992). Such problems can severely limit aquatic habitat, recreational activities, fisheries, and use of a water body as a drinking water supply.

Some species of the bloom-forming cyanobacteria also

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produce toxins, which are released into water primarily following death and senescence of the bloom. Livestock, wildlife, and pet fatalities due to ingestion of water containing toxic cyanobacterial cells or the toxins released by the cells have been extensively documented. Animal poisonings have been reported worldwide, and many more episodes likely go unrecorded or are misdiagnosed (Carmichael 1986, 1994).

Hepatotoxins appear to be the most frequently occurring toxins produced by freshwater algae (Carmichael 1986). First reported to be produced by *Microcystis aeruginosa*, one of the most common bloom-forming species of cyanobacteria, hepatotoxins that consist of seven amino acids are now referred to as microcystins. Microcystins are cyclic peptides that are potent liver toxins, which will cause death in 2–24 h when ingested at a lethal dose. Death results most often from extensive damage to the liver, resulting in massive pooling of blood in the liver and hemorrhagic shock (Carmichael 1994).

The effects of long-term exposure to cyanobacterial toxins on humans are not known. Tumor-promoting activity has been caused by microcystins produced by *M. aeruginosa* (Falconer and Humpage 1996). The extraordinarily high rates of liver cancer in areas of China may be linked to cyanobacterial toxins in local water (Carmichael 1994; Falconer and Humpage 1996). Furthermore, repeated low-level exposure to such toxins could favor development of chronic gastrointestinal and liver disorders (Falconer 1996). Exposure to microcystins at a hemodialysis center in Brazil was believed to have caused the deaths of 50 patients (Jochimsen et al. 1998).

Not all strains of *M. aeruginosa* produce toxins. Although all strains possess genes for peptide synthetases, microcystin-producing and nontoxic strains differ in their content of genes for specific peptide synthetases (Meibner et al. 1996). Therefore, the ability of a strain to produce microcystins may depend on its possession of the gene(s) required. Expression of that gene and toxin production and (or) dominance of a toxic strain in the population may be controlled by environmental conditions.

Environmental conditions may have an effect on the formation of, and short-term changes in, cyanobacterial toxicity observed in natural blooms. Cyanobacterial toxicity may vary widely, between and within lakes, during one sampling season and between years. These unpredictable occurrences may be influenced by lake morphometry, prevailing nutritional factors, and other environmental conditions that affect population growth, accumulation, and dispersal. A variety of factors such as light, temperature, percent DO saturation, nutrient availability, wind patterns, lake mixing, growth stage, and zooplankton grazing are addressed in the literature, with conflicting results reported. Environmental conditions that are required for a toxic bloom to develop are unknown. However, cyanobacterial blooms, whether toxic or not, are generally favored by conditions of (*i*) total phosphorus (TP) content, (*ii*) high temperature levels, (*iii*) high water column stability, (*iv*) low grazing pressure by zooplankton, and (*v*) low nitrogen to phosphorus (N:P) ratios (Paerl 1988, 1996; Hyenstrand et al. 1998).

A toxic *M. aeruginosa* bloom was detected in Steilacoom Lake in western Washington in October 1992. Toxic cyanobacterial blooms in Steilacoom Lake were further investigated in 1994 and 1995 with the following primary objectives: (*i*) to identify and evaluate relationships between environmental factors (e.g., nutrients, water column stability, temperature, light, zooplankton grazing) and toxin occurrence and production and (*ii*) to determine the relationship between the presence and abundance of potentially toxic cyanobacteria and toxin production.

Methods

Study lake

Steilacoom Lake is a small, shallow lake located in Pierce County, Washington, south of Tacoma. The 129-ha lake was created in 1852 by constructing a dam on Chambers Creek and flooding a wetland. It has a volume of 4.3×10^6 m^3 with a maximum depth of 6.1 m and mean depth of 3.4 m. The lake has two inlets, perennial Ponce de Leon Creek and seasonal Clover Creek, and one outlet, Chambers Creek. It is fairly well mixed throughout the year and has an average residence time of 0.10 year. The lake has historically experienced large cyanobacterial blooms and nuisance amounts of aquatic plants. Approximately 80 000 people reside in the lake's 232-km^2 watershed (KCM, Inc. 1996).

Sampling design

Steilacoom Lake was monitored for the occurrence of toxic cyanobacteria blooms and water quality during the summers of 1994 and 1995. Monitoring began 21 July 1994 and occurred approximately once every 1–2 weeks when no toxic bloom was occurring. During the toxic bloom in 1994, monitoring occurred twice a week for about 3 weeks. Station 1, the principal site, was located approximately 0.27 km west of the outlet of Ponce de Leon Creek at a depth of approximately 6 m. In 1994, when a toxic bloom was occurring, samples were collected at both station 1, the deep station, and station 2, which was located on the southern shore. Phytoplankton samples were collected at station 2 from a surface scum that accumulated near the shoreline due to the wind.

Samples were collected between 10 a.m. and 2 p.m. at the surface (depth of 0.5 m) on all 12 sampling occasions in 1994 and on all 17 sampling occasions in 1995. On nine out of 12 sampling visits during summer 1994 and on three out of 17 visits during 1995, samples were collected at discrete depths of 2, 4, and 5.5 or 6 m (approximately 0.5 m from the bottom).

Limnological characteristics

DO and temperature were measured at 1-m intervals at the deep-water station with a YSI model 58 meter, which was calibrated for DO in the laboratory by azide modification of the Winkler method (APHA et al. 1992). Water pH was determined on discrete samples in the laboratory with a Beckman pH meter with a Corning general purpose combination probe. Secchi depth (transparency) was measured at station 1.

Water samples were collected using a 2-L Alpha bottle sampler. Samples were analyzed for total nitrogen (TN), ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), TP, soluble reactive phosphorus (SRP), and chlorophyll *a*. Phosphorus was measured by the ascorbic acid method (APHA et al. 1992). Samples for TP were first digested with persulfate and then analyzed for SRP. TN, NH_4^+-N, and NO_3^--N were analyzed using the automated Korolev, phenate, and cadmium reduction methods, respectively (APHA et al. 1992). Chlorophyll *a* was analyzed using the monochromatic acid-correction method (APHA et al. 1992).

Phytoplankton samples were preserved in the field with approximately 0.4 mL of 1% Lugol's solution. Subsamples of 30 mL were concentrated approximately 10 times by centrifuging the sample at 400 rpm and transferring 3 mL to vials for enumeration.

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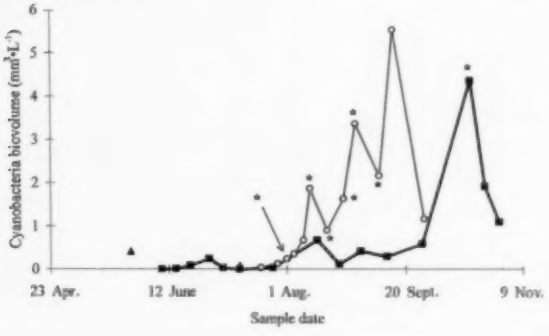
Fig. 1. Biovolume of cyanobacteria during 1994 (circles) and 1995 (squares). KCM, Inc. (1996) data (triangles) were from a composite sample at station 1 during 1994. Asterisks indicate a positive mouse bioassay response.



Phytoplankton were enumerated in a Palmer–Maloney cell (0.1 mL of sample) at 200× magnification. At least 200 Whipple grids were counted in most samples. Unidentified phytoplankton were labeled as unknown and grouped into an appropriate taxonomic class. The average biovolume (cubic millimetres per litre) of each taxon was calculated from the dimensions of representative individual units (cells or colonies) using formulae suggested by Wetzel and Likens (1991). The percentage of total phytoplankton volume was calculated for each genera.

Zooplankton were collected at station 1 on each visit from a vertical net (63 µm) haul from 1 m above the bottom to the water surface. The concentrated sample was preserved with 70% isopropyl alcohol and stored in a 500-mL polyethylene bottle in the dark at room temperature. Subsamples of 5–10 mL of stained zooplankton in dilution water were enumerated in a counting chamber under a dissecting scope. Two or three subsamples were counted and an average of these recorded as the concentration of zooplankton per litre.

Toxicity

Water samples were collected for toxicity analysis by vertical tows to the bottom using a 63-µm zooplankton net to capture and concentrate cyanobacteria. Following collection, samples were delivered to the laboratory for toxicity analysis and taxa identification. Toxicity testing was initially performed using the mouse bioassay (Beasley et al. 1989). Duplicate samples were kept and frozen for later analysis of microcystin if the mouse bioassay showed toxicity.

Selected samples were analyzed for microcystin-LR concentration using high-performance liquid chromatography (HPLC). HPLC has a detection limit of 1 µg·g plankton biomass (dry weight)$^{-1}$ (Kotak et al. 1995). Other microcystins were not identified, but their concentrations were estimated using a microcystin-LR calibration curve. Microcystin concentrations in several samples collected in 1995 were measured using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994) with a detection limit of about 0.25–0.5 µg·g lyophilized sample^{-1}.

Data analysis

Several variables (i.e., lake flushing, zooplankton grazing rate, water column stability) were estimated from the data collected. Monthly flushing rates for 1995 were estimated using ratios of precipitation in 1994–1995 multiplied by measured inflows from 1994 (KCM, Inc. 1996). Zooplankton grazing rates of cubic millimetres of phytoplankton per litre per day were calculated using an assumed average zooplankton filtering rate of 3 mL·zooplankton^{-1}·day^{-1}, multiplied by the concentration of phytoplankton and zooplankton per litre on each sampling date. Relative thermal resistance to mixing (RTRM), an estimate of water column stability, was calculated as the density difference between surface and bottom divided by the density difference between water at 4 and 5°C (Welch 1992).

Chemical constituents were usually uniform throughout the thermally uniform epilimnion so that data from 0.5 m below the water surface were used to relate with cyanobacteria, which tended to concentrate near the surface. For other purposes, field variables (e.g., temperature, pH) were extrapolated to whole-lake, volume-weighted means. The volume-weighting procedure involved determining the percentage of total lake volume represented by each successive depth layer using a bathymetric map. Soluble N (SN) was calculated as the sum of NH_4^+-N and NO_3^--N.

Data collected during May and July 1994 by KCM, Inc. (1996) for the phase I lake restoration study provided supplemental information on lake quality prior to initiation of our monitoring program in mid-July. Analyses for both studies were identical and performed by the same laboratory.

The period from mid-July through the end of September is considered "summer," and values for this period were used in statistical analyses and comparisons between 1994 and 1995. Mean summer values (± 1 SD) at station 1 were compared between years using a two-tailed two-sample t test with a significance level of $p < 0.05$. Data were examined for the assumptions inherent in parametric statistics (e.g., normality, independence, homogeneity of variance) and transformed as appropriate. Cyanobacteria and *M. aeruginosa* abundance, as well as microcystin concentrations, were evaluated relative to the independent variables using a least squares regression technique.

Results

Cyanobacterial abundance

The biovolume of cyanobacteria was much higher, and comprised at least 80%, throughout most of summer 1994 than during 1995 (Fig. 1). Essentially all of the cyanobacteria in the samples from July to mid-September 1994 were *M. aeruginosa* (Fig. 2). Diatoms (Chrysophyta) were the dominant group throughout most of summer 1995. Cyanobacteria did not dominate the phytoplankton community until October 1995. While *M. aeruginosa* was nearly the sole cyanobacterial component in 1994, the most abundant taxon when cyanobacteria dominated in late 1995 was *Aphanizomenon flos-aquae*, with several *Anabaena* species and *M. aeruginosa* being present at lower levels.

Cyanobacterial toxicity

A pronounced and prolonged toxic bloom of *M. aeruginosa* occurred during summer 1994 but not during summer 1995 (Fig. 1). Toxic conditions were detected from early August through October 1994. Total microcystin concentrations ranged from 209 to 1286 µg·g^{-1} at station 1 and from 494 to 1385 µg·g^{-1} at station 2 (Fig. 3). On three dates (21 July, 28 July, and 8 August 1994), toxicity was not detected by mouse bioassay, but high concentrations of microcystins were measured by HPLC on these dates.

Only one sample in 1995 (16 October) was toxic by mouse bioassay; it was composed of *A. flos-aquae*, *Coelosphaerium*, and *M. aeruginosa*. Microcystin concentrations were 77 µg·g^{-1} (cells) and 2000 µg·g^{-1} (supernatant), determined by the ELISA method. A sample collected on 18 October at station 1 was not toxic by mouse bioassay. Microcystin, determined by ELISA, was only 16.7 µg·g^{-1} in

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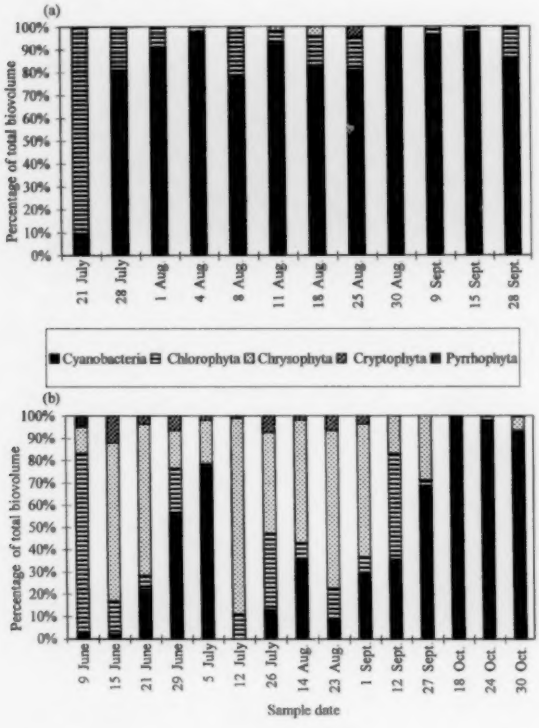
Fig. 2. Seasonal changes in the phytoplankton community composition (percentage of total phytoplankton biovolume) during (*a*) 1994 and (*b*) 1995.



that sample, and a nearshore sample from the same date contained even less microcystin (6.0 $\mu g \cdot g^{-1}$).

Transparency

The lake was less transparent in summer 1994 than in summer 1995 (Fig. 4). Mean Secchi depth transparency (2.8 ± 0.4 m) in 1995 was double that in 1994 (1.4 ± 0.6 m) ($p < 0.05$). There was a significant linear relationship between increasing percent cyanobacteria and decreasing Secchi depth ($r = 0.69$) and a negative exponential relationship between the concentration of cyanobacteria and Secchi depth ($r = 0.82$) (Table 1).

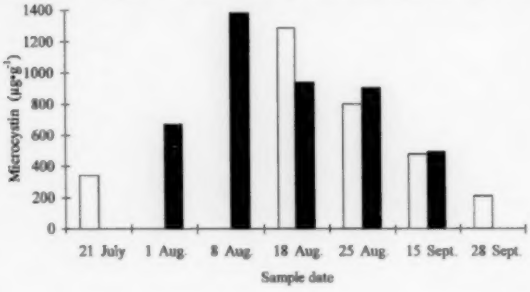
Temperature, DO, and water column stability

Whole-lake, volume-weighted mean water temperature was 18.9°C for both summers. Similarly, temperatures near the lake bottom during the two summers (15–17°C) were not statistically different (Collier 1996). There was, however, a significant difference in average surface water temperature between 1994 (22.6 ± 2.0°C) and 1995 (20.6 ± 1.3°C) ($p < 0.05$) (Fig. 4).

Whole-lake, volume-weighted mean DO concentration was lower in 1994 (7.7 ± 2.2 $mg \cdot L^{-1}$) than in 1995 (9.8 ± 1.4 $mg \cdot L^{-1}$). The lower DO in 1994 was due to a thicker DO-depleted hypolimnion from mid-August through September, indicating stable water column conditions (Collier 1996).

The summer mean RTRM value for 1994 was 188 ± 103, nearly double the mean of 102 ± 40 in 1995 ($p < 0.05$)

Fig. 3. Total microcystin concentrations of total phytoplankton biomass at stations 1 (open bars) and 2 (solid bars) during 1994.



(Fig. 4). RTRM was especially high (>200) between 12 July and 11 August 1994, indicating very stable conditions.

pH

Surface pH values were consistently near 10 from mid-July through September 1994, whereas pH ranged between 8 and 9 during summer 1995 (Fig. 4). The difference between means was significant ($p < 0.05$). Both cyanobacterial and *M. aeruginosa* biovolume were positively correlated with surface pH (Table 1).

Nutrients

Surface TP concentrations in 1994 (42 ± 21 $\mu g \cdot L^{-1}$) were consistently higher than those in 1995 (28 ± 14 $\mu g \cdot L^{-1}$), although the means were not statistically different (Fig. 4). A significant positive relationship between cyanobacteria concentration and surface TP concentration was determined for all samples from both years ($p < 0.0005$) (Table 1). Furthermore, microcystin concentrations were positively correlated with increasing SRP between 1 and 10 $\mu g \cdot L^{-1}$ ($p < 0.05$) (Table 1).

Mean summer TN was significantly higher in 1994 (578 ± 186 $\mu g \cdot L^{-1}$) than 1995 (461 ± 120 $\mu g \cdot L^{-1}$) ($p < 0.05$) (Fig. 4). NH_4^+-N concentrations were higher in 1995 (24 ± 23 $\mu g \cdot L^{-1}$) but not significantly different from those in 1994 (7 ± 5 $\mu g \cdot L^{-1}$). Mean NO_3^--N concentrations were also much higher in 1995 (111 ± 156 $\mu g \cdot L^{-1}$) than those in 1994 (17 ± 22 $\mu g \cdot L^{-1}$), although the difference was not significant. However, *M. aeruginosa* biovolumes were positively correlated with NO_3^--N concentrations ($p < 0.005$) (Table 1).

High cyanobacteria concentrations were associated with low TN:TP ratios (<30) (Fig. 5), and very low cyanobacterial abundance corresponded to high TN:TP ratios (150–160). Additionally, the highest concentrations of microcystins were associated with low SN:SRP ratios (<5), and the lowest microcystin concentration was measured when the SN:SRP ratio was highest (Fig. 6).

Zooplankton

Zooplankton were more abundant in 1995 (>10 · L^{-1}) than in 1994 ($p < 0.05$) (Fig. 7). Zooplankton community composition was also different between summers, with *Daphnia* composing a much greater percentage of the community in 1995 than in 1994 (Fig. 8). Abundance of all genera was low (<5 · L^{-1}) during much of summer 1994, with a peak composed of *Bosmina* in September.

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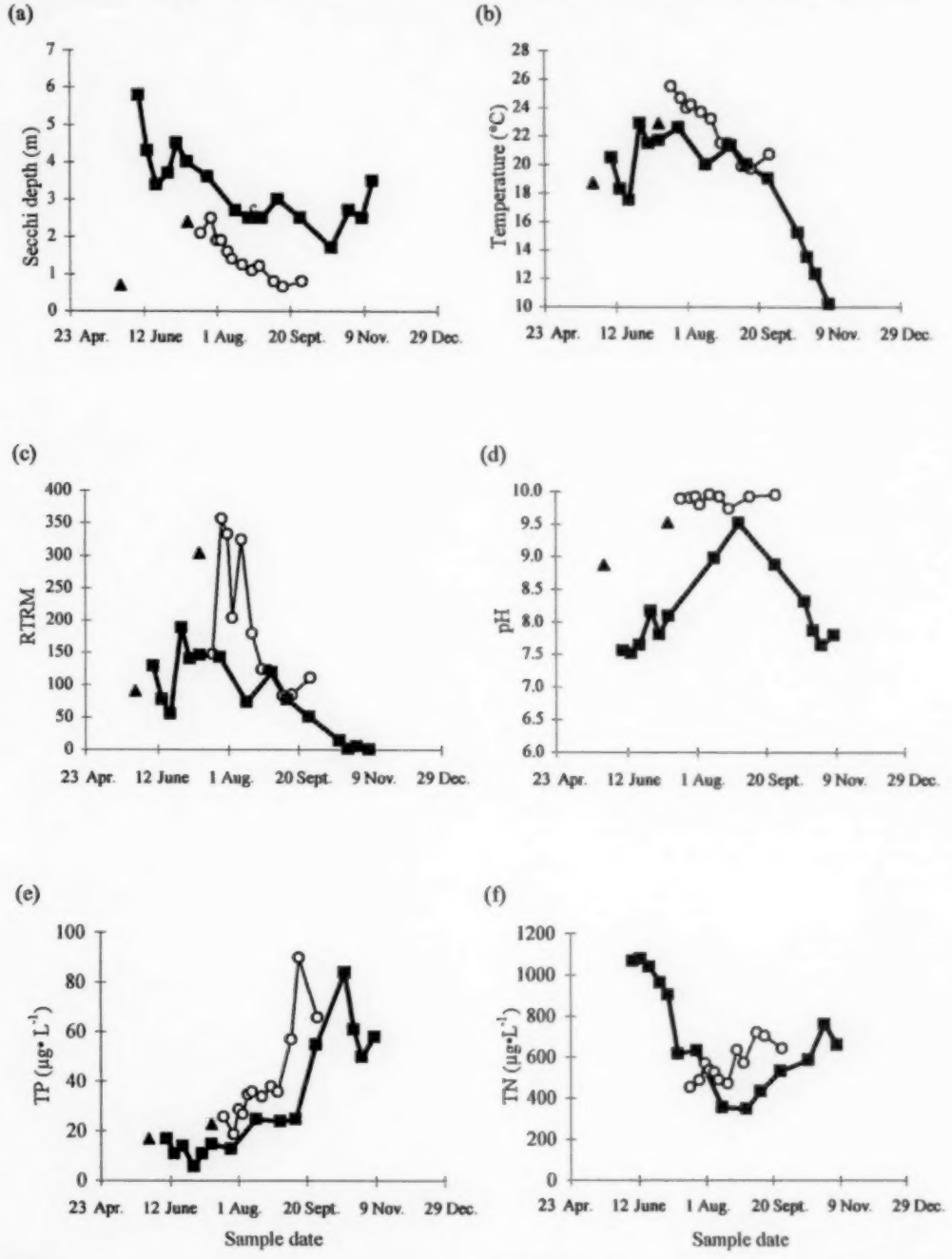
Fig. 4. Surface water column constituents during 1994 (circles) and 1995 (squares). KCM, Inc. (1996) data (triangles) were from a composite sample at station 1 during 1994. RTRM was calculated from density differences between surface and bottom water.



Discussion

The occurrence of a toxic bloom of *M. aeruginosa* one year but not the next permitted comparison of lake conditions between the years and identification of factors that promoted the toxic bloom. Although correlation may not imply cause, the method was used to link physical and chemical factors with (*i*) the development of cyanobacterial dominance in general, (*ii*) the success of *M. aeruginosa* in particular, and (*iii*) the production of microcystins.

Cyanobacterial dominance

Success of cyanobacteria in aquatic systems is attributed to many factors, including their ability to regulate buoyancy

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Table 1. Summary of regression analysis.

Regression	*r*	*p*	*n*
% cyanobacteria = 103.5 – 19.2(Secchi depth)	0.69	<0.001	23
ln[cyanobacteria] = 1.93 – 1.41(Secchi depth)	0.82	<0.0001	23
ln[*Microcystis*] = 1.88 – 1.83(Secchi depth)	0.76	<0.0005	17
% cyanobacteria = –170.0 + 24.4(surface pH)	0.62	<0.005	20
ln[*Microcystis*] = –3.49 + 1.88(surface pH)	0.67	<0.005	15
[Cyanobacteria] = –0.458 + 0.042(surface TP)	0.66	<0.0005	26
ln[*Microcystis*] = –0.899 – 0.007(surface NO_3^--N)	0.64	<0.005	19
[Microcystin] = 199.4 + 100 (surface SRP)	0.96	<0.05	5

Note: *r* regression coefficient; *p*, probability of type I error; *n*, number of data pairs.

Fig. 5. Relationship between cyanobacteria concentrations and volume-weighted TN:TP ratios during 1994 and 1995.

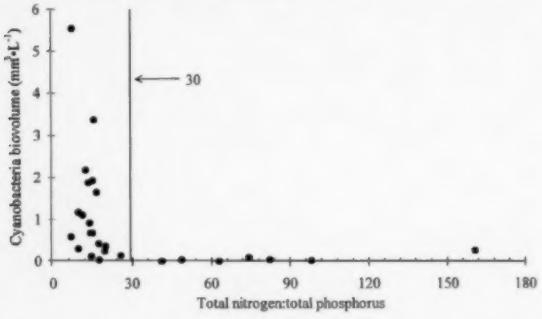
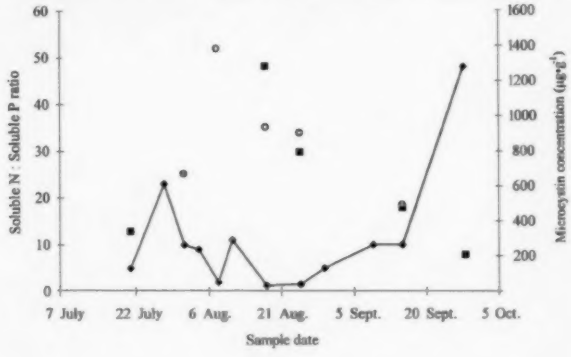


Fig. 6. Relationship between SN:SRP ratios (diamonds) and microcystin concentrations at stations 1 (squares) and 2 (circles) in 1994.



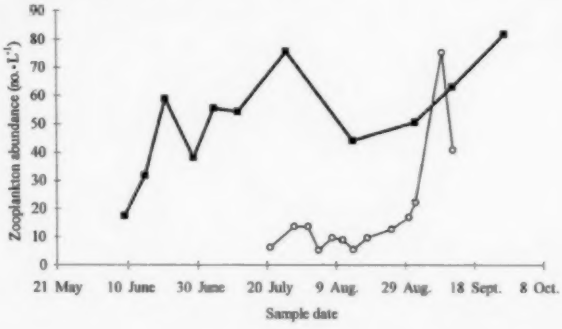
through gas vacuoles, their resistance to grazing by zooplankton due to large size and (or) reduced palatability, their advantage at low CO_2 concentrations and high pH, and their advantage at low N:P ratios (which typically accompany eutrophication) (reviewed by Paerl 1988, 1996; Hyenstrand et al. 1998). A combination, rather than any one of these hypotheses, probably accounts for cyanobacterial dominance. Many of these factors are conditions that cyanobacteria simultaneously create and then use to their advantage (e.g., high pH and low CO_2).

Light, stability, and buoyancy

Cyanobacteria are able to make vertical movements by regulating their buoyancy within the water column through intracellular gas vacuoles (Walsby 1969). This mechanism gives this group of phytoplankton the advantage of locating themselves within a stable water column in a position of optimal light and nutrient availability with the ability to adjust positions as conditions change (Walsby and Booker 1980; Reynolds et al. 1987). Cyanobacterial success is often associated with increased turbidity in the water column that results in light limitation and increased buoyancy (Spencer and King 1987). However, the role of light as a causative factor or one that maintains cyanobacterial dominance is unclear. Low light levels may not contribute to the initial development of cyanobacterial blooms but, instead, as a result of the bloom, may enhance the persistence of cyanobacteria (Blomqvist et al. 1994; Shapiro 1997).

Cyanobacterial blooms in 1994 were associated with decreased water column light intensity and transparency (Secchi depth) and stable water column conditions (RTRM). Stable and turbid conditions during summer 1994 probably

Fig. 7. Zooplankton abundance at station 1 during 1994 (circles) and 1995 (squares).



gave buoyancy-regulating cyanobacteria like *Microcystis* an advantage in obtaining optimum light conditions for photosynthesis. Cyanobacterial biomass suspended in the water column, and concentrated near the surface, would have shaded other phytoplankton below. Cyanobacterial blooms did not occur in summer 1995, probably due partly to a more transparent and less stable water column. Secchi depth values were greater than 2 m from June through September and the mean RTRM values were nearly half those during 1995, indicating less stable conditions.

Trophic interactions

The increased transparency in 1995 may be explained by

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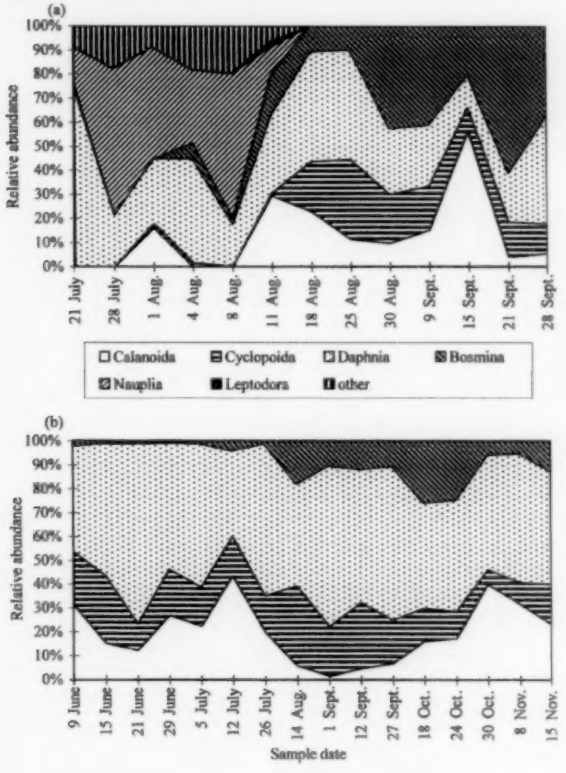
Fig. 8. Seasonal changes in zooplankton community composition (percentage of total zooplankton abundance) during (*a*) 1994 and (*b*) 1995.



trophic-level interactions between planktivorous fish, zooplankton, and phytoplankton. Zooplankton abundance dramatically increased in 1995 concurrent with decreased numbers of young hatchery fish planted in the lake and upstream creeks relative to releases in previous years. Fish hatcheries in the Steilacoom Lake area release surplus coho salmon (*Oncorhynchus kisutch*) fry and fingerlings in nearby lakes and streams in March and April, including Steilacoom Lake, Clover Creek, and Ponce de Leon Creek (Collier 1996). The number of coho fingerlings and fry planted in Steilacoom Lake and upstream creeks in 1995 (201 150) was almost half of the number planted in 1994 (395 050), and the number planted in the lake directly was less than half. The number of fish released in 1993 (434 200) was even higher than in 1994.

Coho young rear in freshwater for more than 1 year before outmigration in early to mid-May through early June. Rearing length achieved depends on their growth rate, which depends on productivity and temperatures in the freshwater system. In lakes, coho may feed on *Daphnia* and fish (Meehan 1991). Salmon planted during both 1993 and 1994 would have had their effect on summer zooplankton abundance in 1994, while fish released in 1994 and 1995 may have affected zooplankton in 1995. Large numbers of young fish planted in 1993 and 1994 could have depleted zooplankton abundance in 1994, while residuals from those releases would have had much less impact in 1995. A conservative number of *Daphnia* eaten in a month by a juvenile fish was estimated at between 17 and 200 (Beauchamp et al. 1995). For a population of approximately 0.4×10^6 young fish, as in 1994, these consumption rates would translate to losses ranging from 6.8×10^6 to 80×10^6 *Daphnia* (approximately 2–20·1000 L^{-1}·$month^{-1}$).

With fewer juvenile fish stocked in the lake and streams in 1995, zooplankton abundance was greater than 40·L^{-1} after June. *Daphnia* was a large part (often >50%) of the zooplankton community that summer. Loss of phytoplankton can be increased by maintaining a large population of large-bodied zooplankton, like *Daphnia* (e.g., Shapiro et al. 1975; Carpenter et al. 1985; Spencer and King 1987). Total phytoplankton losses due to zooplankton grazing and flushing were estimated to be greater during 1995 than during 1994, with losses due to grazing contributing approximately 80% of the total loss (Collier 1996). The large zooplankton population in 1995 likely exerted substantial grazing pressure on phytoplankton, maintaining low biomass through September. With a less stable water column and high transparency (due to zooplankton grazing), low light conditions that favor cyanobacteria did not develop in 1995 until October.

The proposed mechanism by which trophic interactions influence cyanobacterial buoyancy and bloom formation in Steilacoom Lake has been described by Spencer and King (1987). Increased planktivory and the reduced zooplankton abundance may have resulted in greater phytoplankton abundance in 1994. The resulting decrease in light availability, water clarity, and CO_2 availability would have created more favorable conditions for cyanobacterial buoyancy and bloom formation. In 1995, cladocerans could have limited the early-summer development of phytoplankton, resulting in higher water clarity, which would have inhibited cyanobacteria.

Temperature

Cyanobacterial dominance generally occurs at higher water column temperatures (>20°C) (McQueen and Lean 1987). *Microcystis aeruginosa* populations have been shown to succeed at temperatures in the range of 15–25°C (Robarts and Zohary 1987). However, temperature is not considered to be the primary cause for phytoplankton succession but may work in combination with other factors (Welch 1992). In Steilacoom Lake, temperatures exceeded 20°C in both years, but temperatures in 1994 were greater than 22°C more often than in 1995. However, the greater water column stability indicated by higher surface temperature in 1994 was probably more important to cyanobacterial dominance than the slightly greater temperature per se.

Low CO_2 and high pH

At high rates of photosynthesis, phytoplankton deplete CO_2, which causes an increase in pH (Shapiro 1990). Other phytoplankton are not as efficient as cyanobacteria at utilizing CO_2 at low concentrations. Cyanobacteria have lower half-saturation constants (K_s) for CO_2 than other phytoplankton and have the competitive advantage of being able to use both free CO_2 and HCO_3^- as a photosynthetic source of carbon (Talling 1976; Shapiro 1990, 1997). Furthermore, reduced CO_2 stimulates increased buoyancy in cyanobacteria (Booker and Walsby 1981; Klemer et al. 1982; Spencer and King 1985). Like transparency, high pH is an environmental

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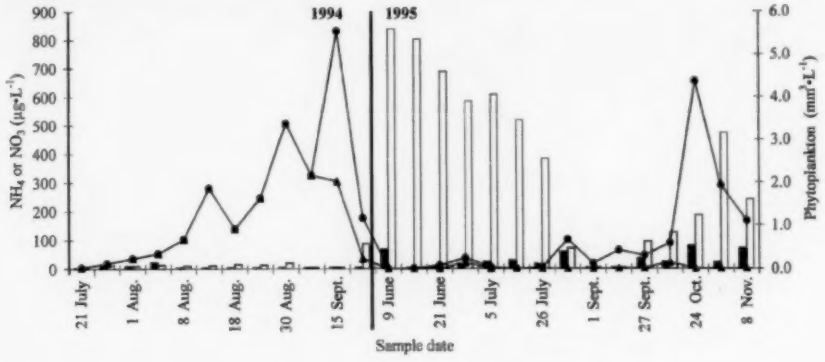
Fig. 9. Comparison of concentrations of cyanobacteria (circles), *Microcystis* (triangles), NO_3^--N (open bars), and NH_4^+-N (solid bars) during the summers of 1994 and 1995.



condition that is partially caused by cyanobacteria and enhances their dominance. Shapiro (1997) concluded that initiation of cyanobacterial development was not caused by high pH or low CO_2 concentration; however, their dominance was maintained by reducing CO_2 to levels lower than those that other phytoplankton could utilize.

Surface pH was much higher in Steilacoom Lake during the toxic bloom in 1994, with values consistently near 10 from mid-July through September. Elevated pH may have contributed to success of cyanobacterial blooms that summer. In contrast, pH values were between 8 and 9 during August and September 1995 when no cyanobacterial bloom occurred until October, at which time the surface pH had dropped to slightly less than 8.

Nutrients

Enrichment with P is usually a precursor to cyanobacterial bloom formation. There are many examples where increased P loading has caused a progression from a clear, less productive state to one that is more turbid and productive and favors nuisance cyanobacterial blooms (e.g., Paerl 1988; Welch 1992). Much higher P concentrations in Steilacoom Lake during summer 1994 probably drove conditions that favored cyanobacterial blooms that year.

The N:P ratio has also been linked to cyanobacterial dominance, along with transparency (Smith 1986) and temperature (McQueen and Lean 1987). The latter authors found that when water temperature was greater than 21°C and the ratio of NO_3^--N:TP was below 5:1, a high probability existed for the occurrence of a cyanobacterial bloom. When temperature was less than 21°C and NO_3^--N:TP greater than 5:1, cyanobacterial blooms did not occur. The NO_3^--N:TP ratio in Steilacoom Lake was less than 5 for all dates sampled in 1994, although the ratio had been higher (about 37) earlier in the year (27 May) (KCM, Inc. 1996). The decrease to below 5 occurred sometime between May and mid-July. Unfortunately, the precise timing of the decline in NO_3^--N:TP ratio in relation to other environmental factors and phytoplankton populations is unknown. Such a decline took over a month in 1995 when the ratio of NO_3^--N:TP dropped to below 5 by around mid-August, more than 1 month later than estimated for 1994, and was followed by a cyanobacterial bloom 1 month later.

Dominance by *Microcystis*

The toxin-producing cyanobacterium *M. aeruginosa* has been present in Steilacoom Lake at least since October 1992 when the first toxic bloom was detected. How a toxic strain of *M. aeruginosa* became established or if a previously nontoxic strain underwent some change to begin producing toxins is unknown. Toxic strains may persist in water bodies in very low concentrations, producing sublethal pulses of toxicity without actual poisoning episodes.

Decreased transparency, stable water column conditions, high TP, reduced grazing, and high temperature and pH would have the same effect in promoting *M. aeruginosa* growth and dominance as for cyanobacteria in general. The success of *M. aeruginosa* over other cyanobacteria may be explained by other environmental conditions.

Blomqvist et al. (1994) proposed that the success of non-N-fixing cyanobacteria (such as *M. aeruginosa*) is influenced by the form of inorganic N present. High NO_3^--N favors the growth of eukaryotic phytoplankton, while low NO_3^--N concurrent with sufficient NH_4^+-N, either present as a pool or recycled at a sufficient rate to supply phytoplankton growth, favors some cyanobacteria. Success of non-N-fixing cyanobacteria was correlated with depletion of NO_3^--N. According to Blomqvist et al. (1994), the ability of some cyanobacteria to succeed is reduced at NO_3^--N concentrations greater than 20 $\mu g \cdot L^{-1}$. Kappers (1984) found that *M. aeruginosa* prefers NH_4^+-N over NO_3^--N as an N source and would be outcompeted by other phytoplankton in high-NO_3^--N environments because of its low NO_3^--N assimilation rate.

Low NO_3^--N concentrations in Steilacoom Lake in 1994, therefore, may partly explain the success of *M. aeruginosa*. There was a dramatic contrast between the amount of *M. aeruginosa*, NO_3^--N, and NH_4^+-N in the lake between 1994 and 1995 (Fig. 9). NO_3^--N was less than the suggested threshold for reduced cyanobacterial success of 20 $\mu g \cdot L^{-1}$ throughout summer 1994, during which time, *M. aeruginosa* persisted as the dominant species. In contrast, NO_3^--N concentrations were much higher (>100 $\mu g \cdot L^{-1}$) during summer 1995 until the beginning of September. The only peak in cyanobacteria biovolume that occurred during 1995 was after NO_3^--N concentrations had dropped below 20 $\mu g \cdot L^{-1}$.

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In addition to the prolonged condition of low NO_3^--N, very low NH_4^+-N concentrations also occurred during 1994. Although NH_4^+-N was lower, supplies may have been adequate through recycling to be used preferentially over NO_3^--N. Extensive decaying mats of *Ceratophyllum demersum* and associated epiphytic green algae floated throughout much of the lake in 1994 but not in 1995 (KCM, Inc. 1996). Recycling from these mats was likely occurring through senescence and decay. Nitrogen is usually released more slowly than P from decaying mats, but most SN is released as NH_4^+ (Landers 1982). This mechanism of N release would have given *M. aeruginosa* a slow but steady supply of NH_4^+-N sufficient for near-maximum growth. Increased NH_4^+-N availability can also stimulate buoyancy in cyanobacteria (Walsby and Klemer 1974; Spencer and King 1985, 1987).

The toxic *M. aeruginosa* episode in 1995 occurred despite conditions that were not entirely favorable for a dominant bloom of this cyanobacterium, although such conditions were more favorable by end of summer – early fall. Detection of toxicity at low biomass suggests that *M. aeruginosa* in Steilacoom Lake may continue to produce toxins to some extent even though abundance is scarce.

Toxin production

Processes leading to increases and decreases in microcystin concentrations in lakes are dynamic, exhibiting changes within and between years, as well as diurnally and spatially (Kotak et al. 1993, 1995). In some of those lakes and dugouts in Alberta (Canada), microcystin-LR concentration was directly related to the biomass of *M. aeruginosa*. However, microcystin concentration in the water column of Steilacoom Lake was not related to *M. aeruginosa* biomass. Others found that maximum toxin concentration does not necessarily correspond to peak concentrations of toxin-producing cyanobacteria (Eloff and van der Westhuizen 1981; Watanabe et al. 1994).

Toxin production per cyanobacteria biomass varied markedly in Steilacoom Lake; microcystin concentrations per gram of phytoplankton increased and then decreased over the period of July–September 1994 (Fig. 3). Toxin content was unrelated to environmental factors except SRP, which showed a clear relationship with the toxin. For the five observations at station 1 with concurrent measurements in 1994, microcystin concentration increased with increasing SRP between 1 and 10 $\mu g \cdot L^{-1}$ (Table 1). Sivonen (1990) found that toxin production by strains of *Oscillatoria agardhii* depended on P. Toxin production by *M. aeruginosa* in Steilacoom Lake may have been similarly limited by P. Microcystins are heptapeptides that require significant cellular energy for synthesis.

In summary, factors that may have promoted cyanobacterial blooms, *M. aeruginosa* dominance, and toxin production were present during one year in Steilacoom Lake, when a prolonged toxic bloom occurred during summer, but were largely absent during another year when toxic conditions did not develop until fall and *Microcystis* was not dominant. Although not necessarily causative, factors that were associated with cyanobacterial blooms in 1994 were high TP, decreased water transparency, high water column stability, high surface water temperature, high pH, and decreased lake flushing. Dominance of *M. aeruginosa* over other cyanobacteria was associated with low N:P ratios and low NO_3^--N with sufficient NH_4^+-N concentrations. Experimental manipulations of environmental factors are needed to further elucidate the mechanisms for toxic bloom formation.

Acknowledgments

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References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF). 1992. Standard methods for the examination of water and wastewater. 19th ed. APHA, AWWA, and WPCF, Washington, D.C.

An, J.S., and Carmichael, W.W. 1994. Use of colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon. **32**: 1495–1507.

Beasley, V.R., Dahlem, A.M., Cook, W.O., Valentine, W.M., Lovell, R.A., Hooser, S.B., Harada, K., Suzuki, M., and Carmichael, W.W. 1989. Diagnostic and clinically important aspects of cyanobacterial (blue-green algae) toxicoses. J. Vet. Diagn. Invest. **1**: 359–365.

Beauchamp, D.A., Riviere, M.G., and Thomas, G.L. 1995. Evaluation of competition and predation as limits to juvenile kokanee and sockeye salmon production in Lake Ozette, Washington. N. Am. J. Fish. Manage. **15**: 193–207.

Blomqvist, P., Pettersson, A., and Hyenstrand, P. 1994. Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. Arch. Hydrobiol. **132**: 141–164.

Booker, M.J., and Walsby, A.E. 1981. Bloom formation and stratification by a planktonic blue-green algae in an experimental water column. Br. Phycol. J. **16**: 411–421.

Carmichael, W.W. 1986. Algal toxins. Adv. Bot. Res. **12**: 47–101.

Carmichael, W.W. 1994. The toxins of cyanobacteria. Sci. Am. **270**: 78–86.

Carpenter, S.R., Kitchell, J.F., and Hodgson, J.R. 1985. Cascading trophic interactions and lake productivity. BioScience, **35**: 634–639.

Collier, D.D. 1996. Factors related to cyanobacterial blooms and their toxicity in two western Washington lakes. M.S. thesis, Department of Civil Engineering, University of Washington, Seattle, Wash.

Eloff, J.N., and Van Der Westhuizen, A.J. 1981. Toxicological studies on *Microcystis*. *In* The water environment: algal toxins and health. *Edited by* W.W. Carmichael. Plenum Publishers, New York. pp. 343–364.

Falconer, I.R. 1996. Potential impact on human health of toxic cyanobacteria. Phycologia, **35**: 6–11.

Falconer, I.R., and Humpage, A.R. 1996. Tumour promotion by cyanobacterial toxins. Phycologia, **35**: 74–79.

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Hyenstrand, P., Blomqvist, P., and Petterson, A. 1998. Factors determining cyanobacterial success in aquatic systems — a literature review. Arch. Hydrobiol. Spec. Issues Adv. Limnol. **51**: 41–62.

Jochimsen, E.M., Carmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., de C. Antunes, M.B., de Melo Filho, D.A., Lyra, T.M., Barreto, V.S., Azevedo, S., and Jarvis, W.R. 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. N. Engl. J. Med. **338**: 873–878.

Kappers, F.I. 1984. On population dynamics of the cyanobacterium *Microcystis aeruginosa*. Ph.D. dissertation, University of Amsterdam, Amsterdam, The Netherlands.

KCM, Inc. 1996. Steilacoom Lake phase I restoration study. Final report. Prepared for Tacoma – Pierce County Health Department by KCM, Inc., Seattle, Wash.

Klemer, A.R., Feuillade, J., and Feuillade, M. 1982. Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria*. Science (Washington, D.C.), **215**: 1620–1631.

Kotak, B.G., Kenefick, S.L., Fritz, D.L., Rousseaux, C.G., Prepas, E.E., and Hudrey, S.E. 1993. Occurrence and toxicological evaluation of cyanobacterial toxins in Alberta lakes and farm dugouts. Water Res. **27**: 495–506.

Kotak, B.G., Lam, A.K.-Y., Prepas, E.E., Kenefick, S.L., and Hrudey, S.E. 1995. Variability of the hepatotoxin, microcystin-LR, in hypereutrophic drinking water lakes. J. Phycol. **31**: 248–263.

Landers, D.H. 1982. Effects of naturally senescing aquatic macrophytes on nutrient chemistry and chlorophyll *a* of surrounding waters. Limnol. Oceanogr. **27**: 428–439.

McQueen, D.J., and Lean, D.R.S. 1987. Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae in Lake St. George, Ontario. Can. J. Fish. Aquat. Sci. **44**: 598–604.

Meehan, W.R. (*Editor*). 1991. Influences of forest and rangeland management on salmonid fishes and their habitats. Am. Fish. Soc. Spec. Publ. No. 19.

Meibner, K., Dittmann, E., and Borner, T. 1996. Toxic and nontoxic strains of the cyanobacterium *Microcystis aeruginosa* contain sequences homologous to peptide synthetase genes. FEMS Microbiol. Lett. **135**: 295–303.

Paerl, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. **33**: 823–847.

Paerl, H.W. 1996. A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. Phycologia, **35**: 25–35.

Reynolds, C.S., Oliver, R.L., and Walsby, A.E. 1987. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. N.Z. J. Mar. Freshwater Res. **21**: 379–390.

Robarts, R.D., and Zohary, T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. N.Z. J. Mar. Freshwater Res. **21**: 391–399.

Shapiro, J. 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verh. Int. Ver. Limnol. **24**: 38–54.

Shapiro, J. 1997. The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. Freshwater Biol. **37**: 307–323.

Shapiro, J., Lamarra, V., and Lynch, M. 1975. Biomanipulation: an ecosystem approach to lake restoration. *In* Water quality management through biological control. *Edited by* P.L. Brezonik and J.L. Fox. Department of Environmental Engineering Sciences, University of Florida, Gainesville, Fla. pp. 85–96.

Sivonen, K. 1990. Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. Appl. Environ. Microbiol. **56**: 2658–2666.

Smith, V.H. 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. Can. J. Fish. Aquat. Sci. **43**: 148–153.

Spencer, C.N., and King, D.L. 1985. Interactions between light, NH_4^+, and CO_2 in buoyancy regulation of *Anabaena flos-aquae* (Cyanophyceae). J. Phycol. **21**: 194–199.

Spencer, C.N., and King, D.L. 1987. Regulation of blue-green algal buoyancy and bloom formation by light, inorganic nitrogen, CO_2, and trophic level interactions. Hydrobiologia, **144**: 183–192.

Talling, J.F. 1976. The depletion of carbon dioxide from lake water by phytoplankton. J. Ecol. **64**: 79–121.

Walsby, A.E. 1969. The permeability of blue-green algal gas-vacuole membranes to gas. Proc. R. Soc. Lond. Ser. B, Biol. Sci. **173**: 235–255.

Walsby, A.E., and Booker, M.J. 1980. Changes in buoyancy of a planktonic blue-green alga in response to light intensity. Br. Phycol. J. **15**: 311–319.

Walsby, A.E., and Klemer, A.R. 1974. The role of gas vacuoles in microstratification of a population of *Oscillatoria agardhii* var. *isothrix* in Deming Lake, Minnesota. Arch. Hydrobiol. **74**: 375–392.

Watanabe, M.F., Park, H.D., and Watanabe, M. 1994. Compositions of *Microcystis* species and heptapeptide toxins. Verh. Int. Ver. Limnol. **25**: 2226–2229.

Welch, E.B. 1992. Ecological effects of wastewater. Chapman and Hall, London, U.K.

Wetzel, R.G., and Likens, G.E. 1991. Limnological analyses. Springer-Verlag, New York.

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Estimates of stream residence time and escapement based on capture–recapture data

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Abstract: The area-under-the-curve method is a widely used method for estimating salmon escapement. The method depends on obtaining an accurate estimate of stream residence time, or stream life. This paper develops an estimator of stream residence time based on capture–recapture data. If the capture–recapture experiment is performed on the entire population, the escapement can also be estimated using the area-under-the-curve method. Simulations showed that the stream residence estimator and the area-under-the-curve estimator provide precise estimates of stream residence and escapement, respectively. These methods were used to estimate the stream residence times and escapements of coho salmon (*Oncorhynchus kisutch*) in a small river on Vancouver Island in 1989 and 1990.

Résumé : La méthode de l'aire sous la courbe est largement utilisée pour estimer les échappées de saumon. Cette méthode exige une estimation précise de la durée du séjour en cours d'eau. Dans le présent article, on élabore un estimateur de la durée du séjour en cours d'eau basé sur des données de capture–recapture. Si les travaux de capture–recapture visent une population entière, alors l'échappée peut aussi être estimée au moyen de la méthode de l'aire sous la courbe. Nos simulations ont montré que l'estimateur de la durée du séjour en cours d'eau et l'estimateur fondé sur l'aire sous la courbe fournissent des estimations précises de la durée du séjour en cours d'eau et de l'échappée, respectivement. Ces méthodes ont été utilisées pour estimer les durées de séjour en cours d'eau et les échappées de saumons cohos (*Oncorhynchus kisutch*) dans une petite rivière de l'île de Vancouver en 1989 et 1990.

[Traduit par la Rédaction]

Introduction

It is important to have accurate and precise estimates of escapement (the number of fish that return to the river to spawn) in order to assess the health of salmon stocks. One widely used method for estimating the escapement is the area-under-the-curve (AUC) method where the estimate is formed as the ratio of the total aggregate residence time (e.g., fish-weeks) experienced by a population divided by the average stream residence time. Separate independent surveys can be used to estimate each component. In this paper, we show how to estimate the stream residence time using a capture–recapture experiment. If this capture–recapture experiment is performed on the entire population of returning salmon, it can also be used to estimate the escapement.

The AUC method has previously been used for measuring fish populations using a constant, predetermined stream residence time. It has been suggested, however, that estimates of stream residence time should be specific to the population being sampled (Perrin and Irvine 1990).

Lady (1996) and Lady and Skalski (1998) reviewed procedures for estimating the stream residence time and developed an estimator based on a single release of marked fish. However, in many situations, fish return to spawn over an extended period, and so the estimate of stream residence time based on a release early in the run may not reflect the population-wide average stream residence time. Our method accounts for the differing numbers of fish returning over the course of the run.

Schwarz et al. (1993) also developed a method to estimate the escapement using a capture–recapture experiment; however, their method does not provide an estimate of the average stream residence time.

In this paper, we develop a method for estimating the average stream residence time based on capture–recapture data. We then show how this estimate can be used to estimate escapement using the AUC method. Properties of the estimator are explored based on simulations and the estimator is compared with the estimator of Lady and Skalski (1998). The performance of our AUC estimator is compared with that of an escapement estimator proposed in Schwarz et al. (1993). Finally, 2 years of experimental data are analyzed.

Materials and methods

Details of the design, required assumptions, and subsequent analysis of certain types of capture–recapture experiments are presented in Seber (1982) and Pollock et al. (1990). At each of s sampling occasions, fish are captured. Sampling is assumed to be instantaneous. Unmarked fish are individually marked and subsequently released. Tag numbers of previously marked fish are recorded and the fish are also released.

The notation used is given in Table 1. There are a total of $3s - 1$ parameters: $p_1, \ldots, p_s$ (the capture probabilities), $\phi_1, \ldots, \phi_{s-1}$ (the survival probabilities), and $B_0, \ldots, B_{s-1}$ (the number of fish that enter between sampling occasions and survive to the next sampling occasion). Schwarz et al. (1993) summarized how to use the Jolly–

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Table 1. Notation.

Fundamental parameters	
s	Number of sampling occasions
t_i	Length of the interval from sampling occasion i to sampling occasion $i + 1$; assume that $t_s = t_{s-1}$
p_i	Probability of capture at sampling occasion i, $i = 1,..., s$
B_i	Number of fish that enter the river system between sampling occasion i and sampling occasion $i + 1$ and *are still alive* at sampling occasion $i + 1$, $i = 0,..., s - 1$; B_0 is defined as the number of fish that enter before the first sampling occasion and are alive at the first sampling occasion
ϕ_i	Probability that a fish alive at sampling occasion i will be alive at sampling occasion $i + 1$, $i = 1,..., s - 1$
Derived parameters	
N_i^-	Number of fish alive in the system immediately before sampling occasion i, $i = 1,..., s$; $N_1^- = B_0$; $N_{i+1}^- = N_i^+\phi_i + B_i$
N_i^+	Number of fish alive in the system immediately after sampling occasion i, $i = 1,..., s$; $N_i^+ = N_i^- - n_i + R_i$; N_i^+ may differ from N_i^- due to losses on capture or injections
B_i^*	Number of fish that enter the system after sampling occasion $i + 1$, $i = 0,..., s - 1$; $B_i^* = B_i \dfrac{\log \phi_i}{\phi_i - 1}$ (derived in Schwarz et al. 1993)
T	Aggregate stream residence time over all spawners
A	Total fish that enter the surveyed stream
R	Average stream residence time
E	Escapement
Statistics	
m_i	Number of marked fish captured in the ith sample, $i = 1,..., s$
u_i	Number of unmarked fish captured in the ith sample, $i = 1,..., s$
n_i	Number of fish captured in the ith sample, $i = 1,..., s$
R_i	Number of the n_i that are released after the ith sample, $i = 1,..., s - 1$
r_i	Number of R_i released at i that are captured again, $i = 1,..., s - 1$
z_i	Number of fish captured before i, not captured at i, and captured later, $i = 1,..., s - 1$

Seber method to estimate these parameters based on the usual summary statistics $\{n_i\}$, $\{m_i\}$, $\{R_i\}$, $\{r_i\}$, and $\{z_i\}$. They also derived the estimator for B_i^* shown in Table 1.

The average stream residence time R is defined as the average time that a fish spends in the system under study (Lady 1996). One possible estimator for R is T/A, where T is the aggregate residence time of all spawners and A is the total number of fish that enter the stream before the last sampling occasion.

The T can be decomposed into two parts: the aggregate time contributed by the N_i^+ fish that were already in the system immediately following sampling occasion i and the aggregate time contributed by the B_i^* fish that entered between sampling occasion i and sampling occasion $i + 1$ (B_i^* may be larger than B_i because some fish could enter between two sampling occasions but not survive to the next sampling occasion).

First consider the contribution by fish already in the system. If such a fish survives until sampling occasion $i + 1$, it contributes an amount of t_i to the aggregate total. If the fish dies before the next sampling occasion, it will, on average, die at the midpoint of the interval (due to the assumption of uniform death on an interval) and contribute an amount of $0.5t_i$ to the aggregate total. Because $N_i^+\phi_i$ of the fish will survive and $N_i^+(1 - \phi_i)$ will die, the overall contribution from fish that were already alive at time i is $N_i^+\phi_i t_i + N_i^+(1 - \phi_i)t_i$.

Because it is relatively easy to extend capture–recapture escapement studies until recruitment has virtually ended, we assume that at time $s + 1$, there are no more fish left alive in the system. This implies that $\phi_s = 0$. Likewise, we assume that at time 0, there are no fish in the system. Then assuming that $t_{s-1} = t_s$, the total contribution is $0.5 \sum_{i=1}^{s-1} t_{i+1}N_i^+(\phi_i + 1) + 0.5\,t_sN_s^+$ units of time.

Second, the B_i^* fish that enter the system between sampling occasions also contribute to the aggregate total between their time of entry and the next sampling occasion, at which time they become part of the contribution above. An individual fish arriving t units after sampling occasion i has a $\phi_i^{(1-t/t_i)}$ probability of surviving until the next sampling occasion and contributes $(t_i - t)$ weeks to the aggregate residence time. We will assume that new fish within each interval arrive uniformly. (Note that we do not assume that the distribution is uniform over the entire experiment; many more fish may arrive in certain intervals than in other intervals.) Integrating over the interval and multiplying by B_i^*, the number of new fish arriving in that interval, the total contribution for that interval is

$$B_i^* \int_0^{t_i} \frac{1}{t_i}(t_i - t)\phi_i^{(1-t/t_i)}\,dt.$$

The fish that arrive before the first sampling time contribute $0.5B_0t_0$ units. The total contribution by newly arrived fish for all intervals simplifies to

$$0.5B_0t_0 + \sum_{i=1}^{s-1} B_it_i\left(\frac{\phi_i}{\phi_i - 1} - \frac{1}{\phi_i}\right).$$

The aggregate resident time is then the sum of both components:

$$T = 0.5\sum_{i=1}^{s-1} t_iN_i^+(\phi_i + 1) + 0.5t_sN_s^+ + 0.5B_0t_0 + \sum_{i=1}^{s-1} B_it_i\left(\frac{\phi_i}{\phi_i - 1} - \frac{1}{\log \phi_i}\right).$$

The total number of fish that entered the stream, A, is found as $A = \sum_{i=0}^{s-1} B_i^*$ where $B_0^* = B_0$. This is the estimator proposed by Schwarz et al. (1993) and would be suitable if the capture–recapture experiment were applied to the entire stream.

The average stream residence is defined as $R = T/A$, and the estimate is found by replacing all parameters by estimates to give

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Table 2. Description of the five simulations used to investigate the effects of violations of the assumptions.

Simulation	Description
Baseline	Using the parameter values given in Table 3
2	Pure heterogeneity in survival rates among fish. As each fish enters the experiment, its survival rate was selected from a uniform distribution on the interval [0.40,0.80]/week and remained at this level until death. Capture rates were homogeneous for all fish as given in Table 3
3	Heterogeneity in survival rates among fish caused by increasing mortality after entering the stream. When fish enter the experiment, they have initial survival rates of 0.60/week. This declines by 20%/week, e.g., fish that entered in week 1 would have a survival rate of 0.48 in week 2; fish that first entered in week 2 would have a survival rate of 0.60
4	Declining survival rate after entry with increased recapture rates for "older" fish. Survival rates decline as in simulation 3, but recapture rates increase as survival decreases, implying that "older" fish are more easily recaptured. The product of survival and recapture was set to 0.25, e.g., a fish with a survival rate of 0.48 would have a recapture rate of 0.52.
5	Pure heterogeneity in recapture rates. As in the baseline simulation, but each fish is assigned a recapture rate from a uniform distribution on the interval [0.05,0.45], which remains fixed for the entire experiment

Table 3. Parameter values used in the baseline simulation.

Sample occasion	New entrants (B_i^*)	Survival rate (ϕ_i)	Capture rate (p_i)
0	100		
1	150	0.60	0.25
2	250	0.60	0.25
3	200	0.60	0.25
4	0	0.60	0.25
5	0	0.60	0.25
6	350	0.60	0.25
7	100	0.60	0.25
8	50	0.60	0.25
9	0	0.60	0.25
10		0.60	0.25
Total	1200		

$$(1)\qquad \hat{R} = \left\{ 0.5\sum_{i=1}^{s-1} t_i \hat{N}_i^{+}(\hat{\phi}_i + 1) + 0.5 t_s \hat{N}_s^{+} + 0.5\hat{B}_0 t_0 + \sum_{i=1}^{s-1} \hat{B}_i t_i \left(\frac{\hat{\phi}_i}{\hat{\phi}_i - 1} - \frac{1}{\log \hat{\phi}_i} \right) \right\} \Bigg/ \left(\hat{B}_0 + \sum_{i=1}^{s-1} \hat{B}_i^{*} \right).$$

The total escapement E in a population can be estimated as

$$(2)\qquad \hat{E} = \widehat{\mathrm{AUC}} / \hat{R}$$

where $\widehat{\mathrm{AUC}}$ is the AUC estimator of the aggregate fish time in the stream (Perrin and Irvine 1990; English et al. 1992). The AUC estimator essentially plots N_i versus time and computes the area under the resulting curve and is found as

$$\widehat{\mathrm{AUC}} = 0.5 t_0 \hat{N}_1^{-} + \sum_{i=1}^{s-1} \frac{t_i}{2} (\hat{N}_i^{+} + \hat{N}_{i+1}^{-}) + 0.5 t_s \hat{N}_s^{+}.$$

The variance of $\hat{R}$ and $\hat{E}$ can be found using a Taylor series expansion of their respective estimators and the variance–covariance matrix of the fundamental parameters. Jackknife and bootstrap procedures can also be used to estimate precision and may perform better when data are sparse, capture rates are low, and the distribution of the estimators is far from normally distributed.

The estimator and its variances have been programmed as part of POPAN (Arnason at al. 1998), an integrated computer system for the analysis of capture–recapture data.

Table 4. Summary of estimates of average stream residence from simulation studies.

	Simulation				
	Baseline	2	3	4	5
Actual stream residence	1.52	1.72	1.08	1.08	1.52
Equation 1	1.70	1.91	1.00	1.08	1.65
Actual SE	0.15	0.20	0.18	0.15	0.18
Average estimated SE	0.21	0.23	0.15	0.14	0.19
Lady and Skalski (1998) estimate	2.06	2.15	1.45	1.08	1.65
Actual SE	0.17	0.54	0.50	0.15	0.18
Average estimated SE	0.63	0.71	0.36	0.33	0.56

Five simulation studies were performed to assess the performance of our estimator. The simulations were conducted as described in Table 2, using parameter values in Table 3 and mimicking those done in Schwarz et al. (1993). The parameter values chosen reflect the observed estimates for the 1989 Chase River study analyzed later in this paper. Each simulation set consisted of 100 simulations.

In the first study, the rate of recruitment to the population and the mortality rate were assumed to be uniform between sampling occasions. As this satisfies the assumptions of our stream residence and escapement estimators, the estimators are expected to perform well. In the remaining four studies, at least one of the underlying assumptions is violated. The extent and types of violations were chosen to be biologically realistic. Note that violations of assumptions about the survival rates will lead to different actual stream residence times, as noted in Table 4.

For each simulated data set, the average stream residence time was estimated using eq. 1 and the maximum likelihood estimator of Lady and Skalski (1998). The escapement was estimated using eq. 2 and the results of Schwarz et al. (1993).

Lastly, the estimators were applied to a study of coho salmon (*Oncorhynchus kisutch*) conducted on the Chase River, British Columbia, in 1989 and 1990. At each weekly sampling occasion, the entire river was sampled by electrofishing. Each fish was tagged and its tag number, sex, degree of sexual maturity, and location of capture were recorded. The experiment was continued until no live fish were observed. The summary statistics for this experiment are given in Schwarz et al. (1993). The average stream residence time was estimated using both eq. 1 and the estimator proposed by Lady and Skalski (1998); escapement was also computed using eq. 2 and the results of Schwarz et al. (1993).

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Fig. 1. Box plots of the estimated stream residence time from the simulation studies centered around the actual stream resident value.

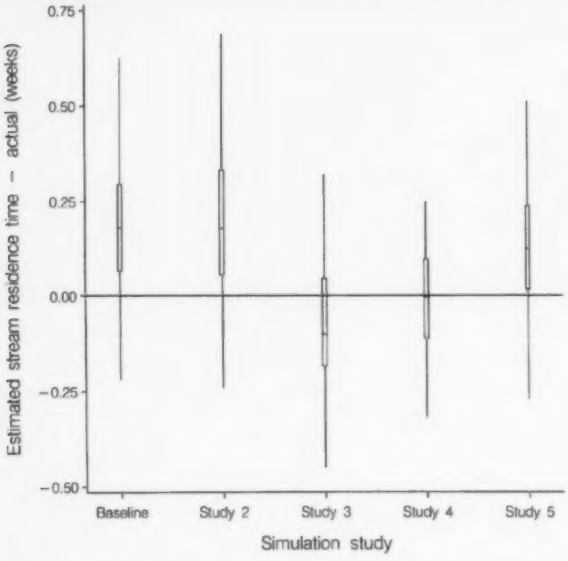
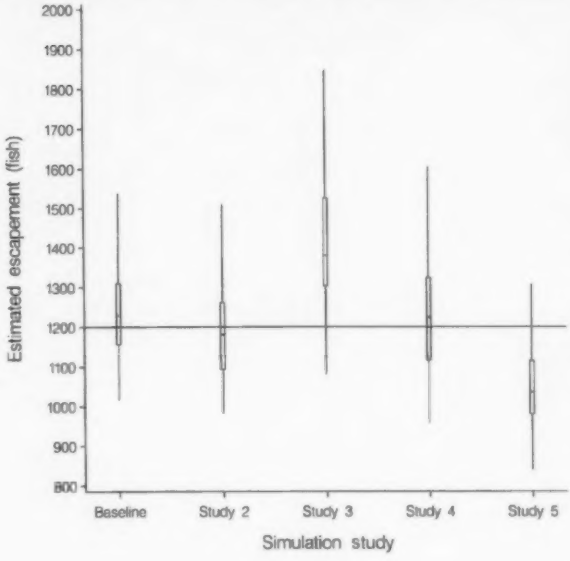


Fig. 2. Box plots of the estimated escapements from the simulation studies. The horizontal line is the actual escapement in the simulation.



Results

Simulation study of stream residence time estimators

Box plots of the estimates from the simulations are shown in Fig. 1 and summarized in Table 4. The box plots show that these estimators have a slightly longer upper tail than if they were normally distributed. This is quite common in capture–recapture estimators, and the extent of the upper tail in the sampling distribution will increase as the capture rates decrease. This nonsymmetric sampling distribution also implies that the usual large sample confidence intervals (estimate ± 1.96 SE) will be shifted downwards compared with the confidence interval found using a bootstrap method.

The estimator performs well on the baseline data; the mean value is only slightly higher than the actual average stream residence time. Because the estimated B_i^*s used underestimate the true values of the new entrants at the beginning and end of the study (Schwarz et al. 1993), the estimated stream residence time has a positive bias on the order of 1 SE.

In simulation 2, in which there was pure heterogeneity in survival rates, the average stream residence time was overestimated. Pollock et al. (1990, section 4.6) suggested that pure heterogeneity in survival rates that is independent of capture rates results in a positive bias in survival rate and population size estimates that would cause the aggregate residence time T to be overestimated. Coupled with the low population estimate on the denominator of the estimator, the stream residence estimate is driven higher. The size of the positive bias for simulation 2 was on the order of 1 SE.

When there is increased mortality of fish after entering the stream, survival rates are biased downwards (Schwarz et al. 1993). This downward bias of $\hat{\phi}_i$ in simulation 3 had the reverse effect of the previous simulation, decreasing the estimates of average stream residence time. The bias is only about 0.45 SE.

There is little difference between the mean stream residence estimate for simulation 3 and that for simulation 4. Because simulation 4 is essentially a repeat of simulation 3 with increasing recapture rates for "older" fish, it would appear that the recapture rate p_i has little effect on the stream residence estimate. The result of simulation 5 provides further evidence that this is the case. Only recapture rate varied between simulation 5 and the baseline simulation. Because both give similar average stream residence estimates, we conclude that the average stream residence estimator performs equally well for homogeneous and heterogeneous recapture rates.

Additional simulation studies (not shown) have demonstrated that as capture rates decrease, the performance of the estimators in the various studies is similar but with a poorer precision.

The actual variation of the estimates matches the average estimate of the SE for all five simulations, indicating that the estimate SE reflects the true variability in the estimator.

Because Lady and Skalski (1998) assumed that all fish are marked only at the beginning of the experiment and assumed that fish arrive only at the midpoint of each interval, their stream residence estimates are biased as shown by the simulation. Note that in simulation 4, fish with lower survival rates are more likely to be captured, which will tend to depress the estimated survival rates in later intervals and reduce the value of their estimates. Box plots indicate that our estimator is less variable than that of Lady and Skalski (1998).

Simulation study of escapement estimators

Box plots of the estimator are shown in Fig. 2 and results summarized in Table 5. Similar comments about the sam-

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Table 5. Summary of estimates of escapement from simulation studies.

	Simulation				
	Baseline	2	3	4	5
Actual escapement	1200	1200	1200	1200	1200
Equation 2	1255	1191	1419	1227	1061
Actual SE	143	111	205	147	126
Average estimated SE	112	105	172	125	91
Schwarz et al. (1993) estimate	1232	1174	1357	1180	1039
Actual SE	140	109	201	142	120
Average estimated SE	133	125	217	162	108

pling distribution of the estimators apply as in the previous section.

The escapement estimator performed very well on the baseline study. The estimator had only a small positive bias, on the order of 0.4 SE. It performed equally well under the assumption of heterogeneous survival rates (simulation 2), with a positive bias of only 0.1 SE.

When survival rates were assumed to be heterogeneous (simulation 3), the estimate of escapement was biased upwards. Because escapement was estimated as AUC/R, this is a direct result of the slight negative bias in the estimate of R, as noted in the previous section. As well, heterogeneous survival rates cause an increase in the estimates of the N_is (Schwarz et al. 1993), resulting in a higher value of AUC. These two factors drive the escapement estimate up. The amount of bias for simulation 3 is only about 1 SE of the actual escapement value.

When both recapture and survival rates were heterogeneous (simulation 4), the estimator performed much better, overestimating the actual escapement by a factor of only 0.2 SE. Because the estimate of R was similar for both simulation 3 and simulation 4, it was the estimate of AUC that was biased downwards in the latter simulation. This is to be expected because increased recapture rates for "older" fish cause a negative bias in the N_is (Schwarz et al. 1993).

In simulation 5, the estimator underestimated the true escapement value. This negative bias was due to two factors. First, as noted above, the estimate of R used in the escapement estimate was too high. Second, as in simulation 4, the AUC value used in the estimate was too low due to the assumption of heterogeneous recapture rates. The error of the AUC estimator in simulation 5 was on the order of 1.1 SE.

Again, the variability in the estimates of escapement matches the average estimates of SE well for all five simulations, indicating that the estimated SE reflects the true variability. The new estimate of escapement performs comparably with that found in Schwarz et al. (1993) with comparable SEs.

Application of the estimators to data from the Chase River in 1989 and 1990

The results are summarized in Table 6. In the 1989 study, most fish appeared to enter after the study had commenced. Consequently, the estimated stream residence time of Lady and Skalski (1998) was based mainly on fish that entered the stream early and would be expected to have a longer stream residence than fish entering later in the run. This leads to the apparent positive bias in their estimator. In the 1990 study, most fish entered early in the study and the two estimators of stream residence time are similar.

Table 6. Estimates of average stream residence and escapement for the Chase River studies.

	1989		1990	
	Estimate (weeks)	Estimated SE	Estimate (weeks)	Estimated SE
Stream residence time				
Equation 1	2.33	0.25	1.49	0.21
Lady and Skalski (1998)	3.23	0.78	1.32	1.31
Escapement				
Equation 2	623	41	1669	163
Schwarz et al. (1993) estimate	587	50	1615	193
Minimum number alive	328		828	

The estimates of escapement for the two years are similar to those reported by Schwarz et al. (1993). The true escapement is unknown, but both estimates are approximately twice the minimum number of unique fish seen.

Discussion

Our stream residence estimator differs from that of Lady and Skalski (1998) in two respects. First, our estimator was developed based on a multiple release of fish whereas that of Lady and Skalski (1998) was based on a single release of marked fish. Because differing numbers of fish may arrive over the course of the run, our more general development may better reflect the true average stream residence time. Second, Lady and Skalski (1998) assumed that new fish always arrive at the midpoint of an interval. Our estimator assumes that fish arrive uniformly within an interval but makes no assumption about uniformity among intervals. Of course, in reality, neither is true, but our assumption is less likely to be violated in practice than the former.

The simulation study showed that our stream residence time estimator was fairly robust to heterogeneity. This is not too surprising, as heterogeneity in survival probabilities does not lead to substantial bias in the estimated survival rates (Pollock et al. 1990).

The simulation study was conducted using an assumed recapture rate similar to that found for the Chase River study. In many cases, the recapture rates will be considerably smaller. However, estimates of survival and population size remain unbiased regardless of recapture rates, so our estimators are expected to remain relatively unbiased, albeit with a poorer precision. A small simulation study could be used to predict the level of precision for a given study.

The AUC escapement estimator was found to give results similar to those of Schwarz et al. (1993). This was to be ex-

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pected because both estimators were developed under the same set of assumptions.

If the same set of capture–recapture data is used to estimate both R and AUC, then any defect in the survey design could be expected to affect both components. However, experience with capture–recapture studies (Pollock et al. 1990) has shown that estimates of survival are robust to violations of assumptions, and as our stream residence time is essentially a weighted average of survival rates, it, too, should be robust. However, capture–recapture studies have shown that population estimates are not robust to failures of assumptions. As AUC is essentially a weighted average of population sizes, it, too, is likely not robust to study failures; nor is the estimate of escapement expected to be robust.

Consequently, it may be advantageous to use two different methods for the two components of the escapement estimate. For example, a capture–recapture study could be used on a small portion of the run (e.g., a tributary), while estimates of the AUC could be obtained for the entire river using more appropriate methods such as an aerial survey.

It should be noted that in Jolly–Seber capture–recapture experiments, not all fundamental parameters may be individually estimable (Schwarz et al. 1993). They also indicated that it is important to either plan the study so that the bias introduced is small or plan the study so that a model that alleviates this confounding (e.g., a model with equal sampling effort) may be fit.

Lastly, comparing model performances using simulated data sets is essential, but so are evaluations based on real data sets and known escapements. Unfortunately, the literature is short of the latter. Joint escapement monitoring and tagging operations have been conducted for several years and at various locations throughout Canada, so there may already be appropriate data sets in existence. Until the models can be tested under real conditions and with known benchmarks, determining which estimator to use will remain largely a matter of conjecture based on the theoretical merits of each estimator.

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References

Arnason, A.N., Schwarz, C.J., and Boyer, G. 1998. POPAN: a data maintenance and analysis system for mark–recapture data. Scientific Report, Department of Computer Science, University of Manitoba, Winnipeg, Man. (Available at http://www.cs.umanitoba.ca/popan)

English, K., Bocking, R.C., and Irvine, J.R. 1992. A robust procedure for estimating salmon escapement based on the area-under-the-curve method. Can. J. Fish. Aquat. Sci. **49**: 1982–1989.

Lady, J.M. 1996. Release–recapture models for estimating the stream residence time of spawning salmon. M.S. thesis, University of Washington, Seattle, Wash.

Lady, J.M., and Skalski, J.R. 1998. Estimators of stream residence time of Pacific salmon (*Oncorhynchus* spp.) based on release–recapture data. Can. J. Fish. Aquat. Sci. **55**: 2580–2587.

Perrin, C.J., and Irvine, J.R. 1990. A review of survey life estimates as they apply to the area-under-the-curve method for estimating the spawning escapement of Pacific salmon. Can. Tech. Rep. Fish. Aquat. Sci. No. 1733.

Pollock, K.H., Nichols, J.D., Brownie, C., and Hines, J.E. 1990. Statistical inference for capture–recapture experiments. Wildl. Monogr. No. 107.

Schwarz, C.J., Bailey, R.E., Irvine, J.R., and Dalziel, F.C. 1993. Estimating salmon spawning escapement using capture–recapture methods. Can. J. Fish. Aquat. Sci. **50**: 1181–1197.

Seber, G.A.F. 1982. The estimation of animal abundance. 2nd ed. Griffen, London, U.K.

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Estimation of juvenile survival, adult survival, and age-specific pupping probabilities for the female grey seal (*Halichoerus gryprus*) on Sable Island from capture–recapture data

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Abstract: We use a longitudinal capture–recapture study from resightings of grey seals (*Halichoerus gryprus*) branded as young on Sable Island to estimate (*i*) the juvenile survival rate from the time of branding to age 4, (*ii*) the yearly adult survival rate from age 4 to age 9, and (*iii*) the age-specific pupping probabilities, i.e., the probability that a seal will first give birth at each age. The estimated juvenile survival rate from branding (just after weaning) to age 4 ranged from 70 to 80%; however, the lower values are known to be biased low because the study was terminated early. The estimated yearly adult survival rates for ages 4–9 ranged from 0.88 to 0.92·year^{-1}. The estimated probabilities of first giving birth to a young seal (pupping) at ages 4–9 are 0.28, 0.41, 0.18, 0.06, 0.05, and 0.02, respectively, and the estimated average age of first pupping is 5.2 years.

Résumé : Nous avons réalisé une étude longitudinale de capture-recapture fondée sur les réobservations de phoques gris (*Halichoerus gryprus*) marqués en bas âge sur l'île de Sable pour estimer (*i*) le taux de survie des juvéniles depuis le marquage jusqu'à l'âge 4; (*ii*) le taux annuel de survie des adultes de l'âge 4 à l'âge 9; et (*iii*) les probabilités de mise bas selon l'âge, c'est-à-dire la probabilité d'une première mise bas à chaque âge. Le taux de survie estimé des juvéniles depuis le marquage (juste après le sevrage) jusqu'à l'âge 4 était de 70 à 80 %, les valeurs les plus basses étant des sous-évaluations parce que l'étude n'a pas duré suffisamment longtemps. Les taux de survie annuels estimés des adultes depuis l'âge 4 jusqu'à l'âge 9 variaient entre 0,88 et 0,92. Les probabilités estimées d'une première mise bas entre les âges 4 et 9 sont respectivement de 0,28, 0,41, 0,18, 0,06, 0,05 et 0,02, et l'âge moyen estimé de la première mise bas est de 5,2 ans.

[Traduit par la Rédaction]

Introduction

The key parameters in studies of population growth are survival, reproductive rates, and ages of first birth. In this paper, we use a longitudinal capture–recapture study based on resightings of grey seals (*Halichoerus gryprus*) branded as young on Sable Island to estimate (*i*) the juvenile survival from the time of branding to age 4, (*ii*) the yearly adult survival rates from age 4 to 9; and (*iii*) the age-specific pupping probabilities, i.e., the probability that a specific-aged seal will give birth for the first time.

Previous studies (e.g., Mansfield and Beck 1977; Harwood and Prime 1978; Hammill and Gosselin 1995) have estimated these rates based on cross-sectional studies. A sample of seals was sacrificed and aged, and an internal examination of the reproductive tracts revealed if the seal had ever bred in previous years. Life table methods were then used to estimate the adult yearly survival rates assuming that the population had a stable age distribution. A simple difference of the proportion of ever-bred seals at each age estimates the individual age-specific pupping proportions. Finally, these two quantities and the rate of increase in the population were used to derive the juvenile survival rates.

Clobert et al. (1994) presented a method to estimate these parameters from a longitudinal capture–recapture study of animals marked as young. In this study design, successive cohorts of animals are marked as young at a known age. Prior to age k at which the youngest individual breeds, animals cannot be observed. Once an animal starts to breed, it may be recaptured or resighted. They fit a Cormack–Jolly–Seber (Cormack 1964; Jolly 1965; Seber 1965) capture–recapture model to the sighting histories. This gives direct estimates of the juvenile survival and adult survival rates, and from changes in the capture probabilities during the accession to breeding status, they estimate the age-specific breeding proportions. Recently, Schwarz and Arnason (2000) developed a method to estimate these parameters from this study design using a new parameterization of an ordinary Jolly–Seber model. Furthermore, they showed that their method avoids many of the problems encountered by Clobert et al. (1994). Because seals have an approximate 12-month gestation period, mating and breeding occurs approximately

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1 year prior to giving birth to a pup, but the methods applied to estimate first breeding times can be used with minor modifications to estimate the first pupping times.

In this paper, we use the method of Schwarz and Arnason (2000) to estimate the survival and pupping rates. We compare our estimates with previously published estimates obtained from cross-sectional studies. Finally, we outline the advantages and disadvantages of the two study designs and discuss some design considerations for the longitudinal study.

Methods

Sable Island is an island off the coast of Nova Scotia, Canada, and is the pupping site of one of the largest breeding colonies of the grey seal in the Northwest Atlantic (Mansfield and Beck 1977). In 1969, 1970, 1973, 1974, 1985, 1986, 1987, and 1989, samples of pups born on the island were sexed and branded with unique brand numbers.

Seals return to Sable Island over a 6-week period starting in mid-December of each year to mate and to give birth. Departures of females from the breeding colony occur over several weeks until about mid-February; the departing females will not rejoin the breeding group until the next year. In each year, several surveys were taken of the island searching for branded seals. The surveys are conducted by a team of four to six observers using small all-terrain vehicles to move among the seals. Each observer is assigned an area of the breeding colony. All adult seals are scrutinized and brand numbers read at distances ranging from 2 to 5 m. The individual numbers are at least 10 cm high on adults and usually are easily discerned at these sighting distances, but errors can occur (Schwarz and Stobo 1999). The first survey each year occurred after some animals had arrived, but few were believed to have departed. Subsequent surveys were approximately 1 week apart. We collapsed the multiple weekly surveys within the first month into a single sighting value for that year. If a seal was sighted in at least one of the weekly surveys, it was treated as seen in that year.

The yearly sighting records define a history vector for each seal indicating when it was born, branded, and subsequently spotted. For example, a history vector 1000011010 for a seal indicates that it was branded in 1985 (the first value that is a 1) and was seen on the breeding grounds in 1990, 1991, and 1993 (presumably to give birth to a pup) but was not seen in 1986, 1987, 1988, 1989, 1992, or 1994. However, just because the seal was "first" sighted on the breeding grounds in 1990, this does not imply that she first gave birth in 1990 (at age 5). She, for example, could have had a first pup at age 4 (in 1989) but because of the timing of the survey, conditions during the survey, other logistical problems, or just by chance was not sighted. The Jolly–Seber model allows for the possibility of these types of unknown events and based on the complete set of capture histories (or the sufficient statistics deduced from the histories) estimates the recapture rates, the interperiod survival rates, and the number of new entrants into the population, in this case, the number of seals giving birth to their first pup. Female seals return both to mate and to give birth, but observers seldom saw a branded female seal without a pup beside her. Consequently, we believe the estimates for pupping probabilities to be largely unbiased by the seals returning without a pup.

As in Clobert et al. (1994), we made the following assumptions in addition to those commonly made in Cormack–Jolly–Seber models. (*i*) All female seals have a zero probability of resighting prior to the year of their first pup. (*ii*) All seals giving birth to pups, whether for the first time or not, have the same probability of resighting. (*iii*) All seals, regardless of whether they are a first-time or a repeat mother, have the same probability of survival in a year. We make no assumptions about the survival rate of seals who have not yet had their first pup until we estimate the juvenile survival rate, at which time, it is assumed that the survival rates prior to first pup are the same as for other seals. (*iv*) All seals in each cohort have pupped by the end of the study. Otherwise, the age-specific estimates will be conditional upon seals having their first pup before the latest age in the study.

The basic principle of the Schwarz and Arnason (2000) method is to ignore the initial capture at birth, treat the first resighting as an initial mark, and treat the second and subsequent resightings (in later years) as recaptures after the initial mark. In this way, the population of seals who have given birth to their first pup is treated as an open population in the Jolly–Seber framework. Seals that pup for the first time are treated as new entrants ("births") into the pupping population. Schwarz and Arnason (1996) parameterized time-specific births in the Jolly–Seber model by the proportion of the total entrants over the course of the study following Crosbie and Manly (1985). This now corresponds directly to the age-specific proportions of seals giving birth for the first time. The adult survival rates are an intrinsic parameter of the Jolly–Seber model and are estimated directly. Lastly, the ratio of the total animals that returned to give birth to the number branded estimates the juvenile survival rate.

Figure 1 illustrates the parameters (recapture, survival, and age-specific pupping proportions) in a Jolly–Seber model of the sighting histories as they would apply in our study for seals branded at birth in 1985–1987 and resighted in 1986 onwards with one additional cohort representing seals from earlier branded cohorts.

Unfortunately, the most general model shown in Fig. 1 is not very useful, as each cohort has its own set of parameters and there is confounding of parameters at the start and end of the study, as listed in Schwarz et al. (1993). This implies that not all parameters are estimable unless further assumptions are made. For example, in Fig. 1, some of the confounded terms are the products $b_{85,4}p_{85,89}$ and $b_{86,4}p_{86,90}$, which imply that $b_{85,4}$ and $b_{86,4}$ cannot be estimated, or the products $\phi_{85,93}p_{85,94}$ and $\phi_{86,94}p_{86,94}$, which imply that the parameters $b_{85,9}$ and $b_{86,8}$ cannot be estimated (see Table 1 for notation).

The easiest way to resolve the confounding at the end of the study is to ensure that the last capture occasion is sufficiently late so that all cohorts have had their first pup. Unfortunately, this is not possible for this study. However, if the yearly survival rate is very high, then the product $\phi_{85,93}p_{85,94}$ essentially measures the final capture rate and the final age-specific pupping proportion can be approximately resolved. As well, models where survival rates are neither time dependent or cohort dependent, e.g., $\phi_{85,93} = \phi_{85,92}$, also can resolve the confounding. (Of course, these models must be consistent with the data at hand; otherwise, other biases are introduced into the estimates.)

Resolving the confounding at the start of the study can be done by assuming that certain parameters are equal across cohorts or by modeling the capture probabilities as functions of covariates. For example, one may be willing to assume that the capture rates are cohort independent, i.e., that $p_{85,j} = p_{86,j} = p_{87,j}$. This would imply that all seals giving birth, regardless of cohort, have the same probability of being recaptured in year j. This would allow the confounding at the start of the study to be resolved. However, the confounding for the first cohort would still be a problem because the first cohort's first capture probability is not shared with any other cohort. However, because we have a separate cohort of seals that have given birth previously that were marked prior to the start of the first recaptures of the new cohorts, this additional cohort could be used to estimate the early capture probabilities (under the assumption of independence of recapture probabilities among cohorts) and this would fully resolve the confounding in all cohorts.

Alternatively, the capture probabilities could be modeled as functions of covariates (as was done in Clobert et al. (1994)), or

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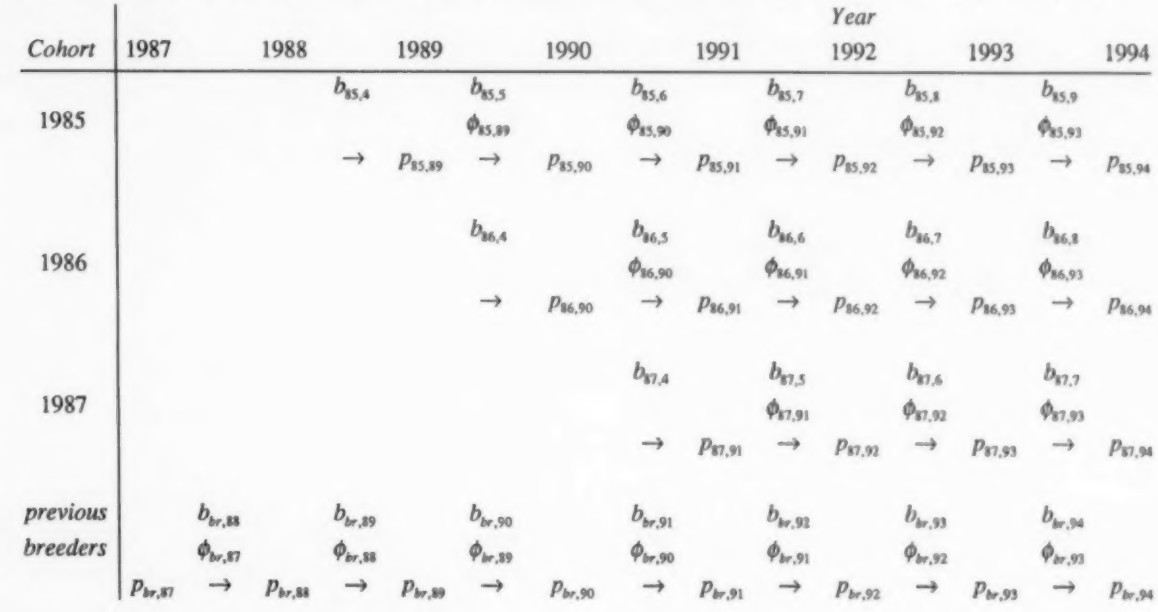
Fig. 1. Representation of the parameters for the four cohorts of grey seals in the study: $p_{i,j}$ is the probability that a seal in cohort i is seen in year j conditional upon it pupping in year j, $\phi_{i,j}$ is the probability that a seal alive in cohort i and in the population of having given birth to a pup in year j is alive and in the pupping population in year $j + 1$, and $b_{i,a}$ is the probability that a seal from cohort i will give birth for the first time at age a. Note that the last cohort consists of a combination of many previous cohorts, and hence, the $b_{i,a}$ should be interpreted as the proportion of animals from previous cohorts who give birth for the first time in calendar year a.



possibly, it may be tenable to consider models where the capture probabilities are constant over time.

There could be a large number of possible models that will have to be considered in reference to the data at hand. For example, the capture rates could be cohort independent; survival rates year independent, etc. Some of the more interesting submodels are those where the last age of first pupping is successively reduced to attempt to identify the age by which all animals have given birth to their first pup. Another interesting set of models is where the age-specific pupping proportions are the same for all cohorts.

Burnham and Anderson (1998) suggested that model selection be done using both formal likelihood ratio tests and consideration of the Akaike information criterion (AIC) for the various models. The latter attempts to measure both the fit of the model and the number of parameters needed to describe the model, and models with similar AIC values should be considered as fitting equally well. The various Jolly–Seber models described below were fit to the data using the methods outlined in Schwarz and Arnason (1996) and the computer package POPAN (Arnason et al. 1998) based on the usual summary statistics for capture–recapture data.

Following the model fit, the average age of first pupping was estimated as

$$\hat{A}_i = \sum_{j=1}^{m} j\hat{b}_{i,j}$$

and the estimated standard error was found using a Taylor-series expansion and the estimated variance–covariance matrix of the age-specific estimates.

The juvenile survival rate is estimated by extrapolating the number of seals returning to give first birth at each age back to age 4 assuming that the survival rates from age 4 onwards are applicable to these seals, i.e., for cohort i:

$$\hat{\theta}_{i,0-4} = \frac{[\hat{B}_{i,4} + \hat{B}_{i,5}/\hat{\theta}_{i,4} + \hat{B}_{i,6}/(\hat{\theta}_{i,4}\hat{\theta}_{i,5}) + \ldots]}{N_i}$$

Here, $\hat{\phi}_{i,a}$ refers to the yearly survival rate for a seal of age a from cohort i, which is found directly from year-specific survival rates ($\hat{\phi}_{i,j}$). The estimated standard error is again found by a Taylor-series expansion.

Results

The usual Jolly–Seber summary statistics for the 1985–1987 branded cohorts and a fourth cohort consisting of all branded seals from previous cohorts are shown in Table 2.

Female seals were first observed returning to the breeding grounds to give birth at age 4. Because no females were observed giving birth before age 4, some of the rows of the summary statistics are zero. This is strictly an artifact of the way the data are presented for modeling — it is necessary to have the same sampling occasions available for all cohorts — but it is a simple matter to constrain the sighting rates to be zero in these cases.

Because no seals were observed giving birth prior to age 4, the age-specific pupping rates are assumed to be zero for ages 1–3. Unfortunately, this study was terminated in 1994 (at age 9 for the 1985 cohort). Consequently, all estimates are conditional upon seals first giving birth at or before age 9. Fortunately, Hammill and Gosselin (1995) found no evidence of first pupping after age 9 based on their cross-sectional studies, so we expect any biases resulting from this conditioning to be slight.

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Table 1. Notation used in the paper. In this study, there are G cohorts of animals marked as young (for simplicity, at age 0).

Parameters	
b_{ia}	Probability that an animal in cohort i that survives until it gives birth for the first time will give birth at age a; $i = 1,..., G$; $j = 1,..., m$; $\sum_{a=1}^{m} b_{ia} = 1$
B_{ia}	Number of animals in cohort i that survive until they give birth for the first time at age a; $i = 1,..., G$; $j = 1,..., m$
ϕ_{ij}	Probability that an animal in cohort i that is alive in year j and has given birth at least once will be alive in year $j + 1$; $i = 1,..., G$; $j = i,..., T - 1$
$\phi_{i,0-4}$	Probability that an animal in cohort i that was branded will survive to age 4. This is the juvenile survival rate
p_{ij}	Probability that an animal in cohort i that has previously given birth or that will give birth in year j will be recaptured in year j; animals that have not previously given birth or are not giving birth have a zero probability of being recaptured; $i = 1,..., G$; $j = i,..., T$
A_i	Average age of first pupping for cohort i; $A_i = \sum_{j=1}^{m} jb_{i,j}$
Statistics	
N_i	Number of animals branded as young in cohort i
n_{ij}	Number of animals in cohort i, marked at age 0, that are recaptured in calendar year j; $i = 1,..., G$; $j = i,..., T$
m_{ij}	Number of animals in cohort i, marked at age 0, that are captured in year j and were previously recaptured prior to year j; $i = 1,..., G$; $j = i,..., T$
R_{ij}	Number of animals in cohort i, marked at age 0, recaptured in year j and released in year j; R_{ij} may differ from n_{ij} because of losses on capture or injections; $i = 1,..., G$; $j = i,..., T$
r_{ii}	Number of animals in cohort i, marked at age 0, from R_{ij} that are subsequently recaptured after release; $i = 1,..., G$; $j = i,..., T$
z_{ij}	Number of animals in cohort i, marked at age 0, recaptured before year j, not recaptured in year j, and recaptured after year j; $i = 1,..., G$; $j = i,..., T$

Note: For convenience, we assume that animals give birth for the first time at age 1, that all animals have given birth for the first time by age m, that successive capture occasions are 1 year apart, and that observations on the population start in calendar year 1, the first year when the first cohort gives birth for the first time. Observations continue until calendar year T with $T \geq m$.

An initial model was fit allowing for each of the four cohorts to have their separate resighting, survival, and age-specific pupping proportions (log-likelihood value −1674.1 with 57 parameters and AIC 3462.2). Each cohort was also subjected to the four goodness-of-fit tests described by Pollock et al. (1990) and Pradel (1993). There was no evidence of lack of fit except for some evidence that animals seen for the first time in any year (i.e., first time breeders) are somewhat less likely to be seen in subsequent years compared with animals first seen in previous years. A plot of the estimated recapture rates (Fig. 2) showed that these rates are similar, but there is some evidence of differential sighting rates. For the 1985 and 1986 cohorts, there was some additional effort expended outside the weekly sighting trips to locate animals of these cohorts for other studies, but the current data records and field notes do not indicate if this additional effort was included in the sighting records. Unfortunately, this model does not allow estimation of all the age-specific pupping proportions, and so, some restrictions need to be applied to the resighting rates to allow estimability. A second model was fit where resighting rates were independent of cohort (log-likelihood value −1683.5 with 48 parameters and AIC 3463.0). A likelihood ratio test between these two models shows some evidence that this simpler model may not be tenable ($\chi^2 = 18.8$, 9 df, $p = 0.02$), but the AIC values are virtually the same so that either model may be acceptable. The significance level may reflect the large power in this study with the large sample sizes present. Models with a constant cohort-specific annual adult survival rate were also tenable (log-likelihood value −1687.7 with 39 parameters and AIC 3453.4).

The estimated age-specific pupping proportions and average age of first giving birth for this last model are shown in Table 3. Note that because of the termination of the study in 1994, the 1986 and 1987 cohorts did not reach age 9, and so the estimates are conditional upon first pupping at or before the last sightable age.

The average annual yearly survival rate is very close to 0.90·year^{-1}. Approximately 70–80% of seals survive from the time of branding to age 4. However, because the study was terminated in 1994, the juvenile survival estimates for the 1985 and 1987 cohorts are biased downwards because some seals in this cohort have not yet returned to give birth to their first pup and would not have been included.

A fourth model in which the age-specific pupping proportions were assumed to be equal in the 1985–1987 cohorts was fit (log-likelihood value −1695.05 with 39 parameters and AIC 3468.1) giving the estimates shown in Table 2. Note that because the latter two cohorts were not observed until age 9, they contributed information only on the age of first pupping for the earlier ages. When compared with the second model, the likelihood ratio test ($\chi^2 = 23.06$, 9 df, $p = 0.006$) indicated that this third model did not fit well compared with the second model and the change in AIC was reasonably large. Nevertheless, this provides a reasonable summary of the age-specific pupping proportions and the average age of first birth from the three cohorts.

Discussion

Hammill and Gosselin (1995) reported mean ages of first birth ranging from 3.61 (SE 0.76) to 5.06 (SE 0.12) years, as

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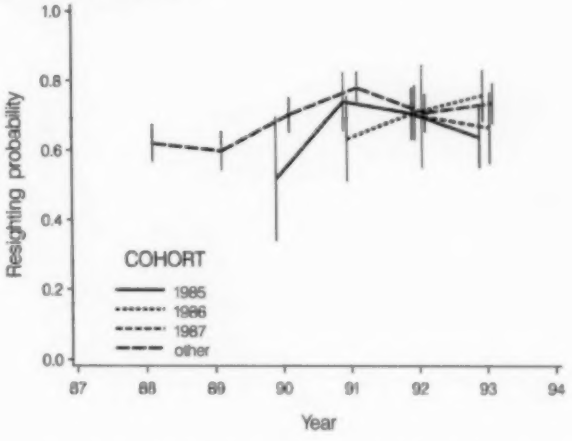
Table 2. Summary statistics for the 1985–1987 branded cohorts of female grey seals and a fourth cohort consisting of all previously branded seals.

Resighting year j	n_{ij}	m_{ij}	R_{ij}	r_{ij}	z_{ij}
Seals branded in i = 1985; total branded 400					
1987	0	0	0	0	0
1988	0	0	0	0	0
1989	35	0	35	32	0
1990	114	17	114	108	15
1991	180	95	180	152	28
1992	174	137	174	132	43
1993	155	131	155	92	44
1994	146	136	146	0	0
Seals branded in i = 1986; total branded 400					
1987	0	0	0	0	0
1988	0	0	0	0	0
1989	0	0	0	0	0
1990	75	0	75	67	0
1991	152	45	152	129	22
1992	163	113	163	134	38
1993	162	141	162	113	31
1994	159	144	159	0	0
Seals branded in i = 1987; total branded 400					
1987	0	0	0	0	0
1988	0	0	0	0	0
1989	0	0	0	0	0
1990	0	0	0	0	0
1991	52	0	52	45	0
1992	131	34	131	99	11
1993	141	83	141	92	27
1994	151	119	151	0	0
Seals branded in i = all previous years					
1987	514	0	514	366	0
1988	252	234	252	232	132
1989	252	229	252	221	135
1990	274	258	274	245	98
1991	295	276	295	254	67
1992	240	237	240	208	84
1993	241	231	241	178	61
1994	248	239	248	0	0

determined by examining the corpora lutea in the wall of the uterus, and from 5.03 (SE 0.22) to 6.08 (SE 0.32) years, as determined by examining for the presence of a fetus. Our results are comparable. Their estimated age-specific first pregnancy proportions were 0.01, 0.44, 0.45, and 0.10 for female seals aged 3, 4, 5, and 6+ years, respectively, as determined by examining the corpora lutea, and 0.18, 0.68, 0.02, and 0.12 for female seals aged 4, 5, 6, and 7+ years, respectively, as determined from the presence of a fetus. Harwood and Prime (1978) estimated these proportions as 0.16, 0.45, and 0.39 for ages 5, 6, and 7+, respectively. Mansfield and Beck (1977) obtained values of 0.16, 0.55, 0.18, and 0.11 for ages 4, 5, 6, and 7+, respectively. Our results are comparable with these.

The greatest value of our analysis is that it provides rates based on successful pregnancies. This could be particularly important for younger animals for which ovulation but no fertilization occurs or for which spontaneous abortion is prevalent.

Fig. 2. Plot of the estimated resighting probabilities from the full model allowing grey seal each cohort to have its own parameters. Note that in the full model, the resighting probabilities are confounded for the first and last year of sightings and cannot be individually estimated.



The life table methods of Mansfield and Beck (1977) and Harwood and Prime (1978) gave estimated annual adult survival rates of 0.87 and 0.94·year^{-1}, respectively. Zwanenburg and Bowen (1990) estimated that the survival rate was 0.96·year^{-1} in order to obtain a 12.6% annual increase in pup production. Our estimates are again similar.

The life table approaches cannot unambiguously estimate juvenile survival rates, as these are determined by the requirement that the population growth from the derived Leslie matrix match the observed population growth. Harwood and Prime (1978) found that the juvenile survival rate could range from 0.41 to 0.61 in their model and arbitrarily chose the value 0.49. Zwanenburg and Bowen (1990) used a value of 0.79 in their model. On the surface, our estimates appear to be much higher; however, our estimates exclude mortality from birth to the time of branding, which cannot be estimated from this study. Consequently, our estimates cannot be used directly in a Leslie matrix without this further information. The estimates are consistent with the lower bound derived from the minimum number of seals alive (MNA = $\Sigma(n_i - m_i)$), which range from 0.60 to 0.72. The minimum number alive is known to be an underestimate of the total number alive, as it does not account for the seals alive but not seen and consequently provides a lower bound to the juvenile survival rate.

Similar methods could be used to estimate the population parameters for the males. However, "first breeding" must be carefully defined for a male, as during the intervening period between sexual and social maturity, these males are forced to the periphery of the breeding colony, and infrequently observed, and only occasionally succeed in copulating with females (Hewer 1961; Godsell 1991). As such, first breeding could be defined as first attaining social dominance. Unfortunately, the current study was terminated before sufficient data could be accumulated on the males.

Our proposed method has a number of advantages over the longitudinal methods used by Clobert et al. (1994), as discussed by Schwarz and Arnason (2000). In particular, the

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Table 3. Estimates of the age-specific grey seal pupping proportions from the 1985–1987 cohorts when a model is fit with separate proportions for each cohort and when a common model is fit to all cohorts.

	Model allowing for separate age-specific pupping proportions for each cohort						Model with common proportions for all cohorts	
	1985 cohort		1986 cohort		1987 cohort			
	Estimate	Estimated SE	Estimate	Estimated SE	Estimate	Estimated SE	Estimate	Estimated SE
Juvenile survival ($\phi_{i,0-4}$)	0.83	0.04	0.77[a]	0.03	0.70[a]	0.02		
Adult survival (ϕ_i)	0.92	0.014	0.91	0.017	0.88	0.03		
Age of first pup								
4	0.19	0.032	0.39	0.041	0.26	0.033	0.28	0.021
5	0.40	0.047	0.39	0.048	0.47	0.047	0.41	0.027
6	0.23	0.044	0.14	0.038	0.19	0.045	0.18	0.023
7	0.09	0.031	0.04	0.026	0.08	0.033	0.06	0.015
8	0.07	0.025	0.04	0.020	Unknown[b]	—	0.05	0.013
9	0.02	0.018	Unknown[b]	—	Unknown[b]	—	0.02	0.011
Average age	5.5	0.09	4.9	0.082	5.1	0.076	5.2	0.061
Log-likelihood value				−1687.7			−1695.05	

[a]Because the study was terminated early, these values will be biased downwards. Refer to text for more details.
[b]Because the study was truncated before this age-class recruited, it is impossible to estimate the proportion for this age-class and the estimates in its column are conditional upon first pupping at or before the last observable age.

method is much simpler conceptually and does not require a complex model that tries to account for the heterogeneity in sighting rates caused by pooling both breeders and nonbreeders. Pradel (1996), Pradel et al. (1997), and Pradel and Lebreton (1999) used a slightly different method to estimate the age-specific breeding proportions based on reading the capture histories "backwards" in time. However, as shown in Schwarz and Arnason (2000), Pradel's age-specific breeding proportions are conditional upon animals surviving until the age at which all animals have become breeders. For long-lived animals, his estimates of the age-specific breeding proportions should be very similar to our estimates; however, for short-lived animals, his method will tend to overestimate the proportion in the older age-classes and underestimate the proportion in the younger age-classes, which will lead to a positive bias in the estimate of the average age of breeding.

There are a number of advantages and disadvantages to cross-sectional versus longitudinal designs. In a cross-sectional study, it is only necessary to sample in one year, so results can be obtained relatively quickly. However, a key assumption is that the seals of each age-class are a random sample of all seals alive at that age. If nonbreeders are essentially uncatchable, then it may be difficult to obtain such a sample. For example, if sampling were restricted to Sable Island in January and February, then only seals giving birth would be sampled and a cross-sectional study would not be valid. In a longitudinal study, animals that have a very long breeding entry curve will require a study that spans many years. This may be too costly. Furthermore, either another set of known breeders must be additionally marked or some additional assumptions about the equality of parameters across cohorts must be made to ensure that parameters are identifiable. In a cross-sectional study, the seal must be sacrificed to determine if she has previously given birth and to age the animal; this may not be acceptable for endangered populations or may not be wise from a public relations viewpoint. In the longitudinal study design, the marked animal only need to be individually observable and breeders distinguishable from nonbreeders. In this study, we are making the explicit assumption that a seal first returns to Sable Island only when ready to give birth, and hence, any seal observed will be pupping. This assumption is reasonable for Sable Island grey seals because these mothers stay with their pups continuously throughout the lactation period (Bowen et al. 1992). It is not necessary to assume site fidelity. If site switching (e.g., between the Gulf and Sable islands) occurs, then as long as the temporary emigration is random (i.e., does not depend on what occurred in previous years), this just reduces the effective sighting probabilities but does not affect survival or reproductive estimates (Burnham 1993). Of course, if no animals have been previously tagged, then a longitudinal study is impossible and a cross-sectional study may be the only feasible study. While advances in DNA technology or self-marking schemes (e.g., individuals identifiable from photographs) may make it feasible to conduct a longitudinal study, achieving the necessary sample size would be problematic.

It is also difficult to envision how a cross-sectional study could be used to investigate the age of first social dominance for males. In theory, this could be attempted using our methods in a longitudinal study.

Lastly, in all longitudinal studies of this type, there are several issues that must be carefully considered during the design phase. First, the study must be long enough that all animals in at least one cohort enter breeding status before the end of the study. Otherwise, the estimates of the age-specific breeding proportions will be conditional upon entering breeding status by the end of the study. Our study suffers from this flaw, but we do not believe that it causes serious bias because previous work has shown that most seals have given birth to their first pup by age 9. Second, great care

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must be taken to properly define the study population and to ensure that this definition does not change over time. For example, if the area searched for seals giving birth changes over time, then the estimates of the age-specific pupping proportion will be confounded with changes in the study area because recruitment is now confounded with population changes in the different-sized study area. In our case, this was not a problem because Sable Island is relatively small and all breeding areas, both old and new, were easily discerned by both aerial and ground preliminary surveys. Although the population on Sable Island has been increasing at a rate of about 12%·year^{-1}, with both expansion of the geographic area occupied by existing breeding concentration and development of new areas (see Stobo and Zwanenburg 1990), the survey design was expanded with the population increase to ensure that all breeding areas were surveyed with equal intensity throughout the study period. Third, because these estimates are directly related to estimates of "births" in the ordinary Jolly–Seber method, all of the problems associated with heterogeneity in capture probabilities and bias in the estimate of birth" will also be important here. Because numerous trips were made within each study year and the same trained observers were used, we do not feel that heterogeneity of sighting probabilities is a serious problem. Fourth, a sufficiently large number of animals must be marked as young to ensure that adequate sample sizes will be observed from the subsequent breeders. In this study, we were fortunate that many seals were branded as young; a simulation study could be used to examine if a proposed program leads to estimates that are sufficiently precise to be useful.

Acknowledgments

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References

Arnason, A.N., Schwarz, C.J., and Boyer, G. 1998. POPAN-5: a data maintenance and analysis system for mark–recapture data. Scientific Report, Department of Computer Science, University of Manitoba, Winnipeg, Man. (Available at http://www.cs.umanitoba.ca/ ~popan)

Bowen, W.D., Stobo, W.T., and Smith, S.J. 1992. Mass changes of grey seal *Halichoerus grypus* pups on Sable Island: differential maternal investment reconsidered. J. Zool. (Lond.), **227**: 607–622.

Burnham, K.P. 1993. A theory for the combined analysis of ring-recovery and recapture data,. *In* Marked individuals in the study of bird populations. *Edited by* J.-D. Lebreton and P.M. North. Birkhauser-Verlag, Basel. pp. 199–213.

Burnham, K.P., and Anderson, D.R. 1998. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.

Clobert, J., Lebreton, J.-D., Allaine, D., and Gaillard, J.M. 1994. The estimation of age-specific breeding probabilities from recaptures or resightings in vertebrate populations: II. Longitudinal models. Biometrics, **50**: 375–387.

Cormack, R.M. 1964. Estimates of survival from the sighting of marked animals. Biometrics, **51**: 429–438.

Crosbie, S.F., and Manly, B.F.J. 1985. Parsimonious modelling of capture–mark–recapture studies. Biometrics, **41**: 385–398.

Godsell, J. 1991. The relative influence of age and weight on the reproductive behaviour of male grey seals *Halichoerus Grypus*. J. Zool. (Lond.), **224**: 537–551.

Hammill, M.O., and Gosselin, J.F. 1995. Grey seal (*Halichoerus grypus*) from the Northwest Atlantic: female reproductive rates, age at first birth, and age of maturity in males. Can. J. Fish. Aquat. Sci. **52**: 2757–2761.

Harwood, J., and Prime, J.H. 1978. Some factors affecting the size of the British grey seal population. J. Appl. Ecol. **15**: 401–411.

Hewer, H.R. 1961. The determination of age, sexual maturity, longevity, and a life table in the grey seal (*Halichoerus grypus*). Proc. Zool. Soc. Lond. **142**: 593–624.

Jolly, G.M. 1965. Explicit estimates from capture–recapture data with both death and immigration — stochastic model. Biometrika, **52**: 225–247.

Mansfield, A.W., and Beck, B. 1977. The grey seal in eastern Canada. Mar. Ser. Tech. Rep. No. 704.

Pollock, K.H., Nichols, J.D, Brownie, C., and Hines, J.E. 1990. Statistical inference for capture–recapture experiments. Wildl. Monogr. No. 107.

Pradel, R. 1993. Flexibility in survival analysis from recapture data: handling trap dependence. *In* Marked individuals in the study of bird populations. *Edited by* J.-D. Lebreton and P.M. North. Birkhauser-Verlag, Basel. pp. 29–37.

Pradel, R. 1996. Utilization of capture–mark–recapture for the study of recruitment and population growth rates. Biometrics, **52**: 703–709.

Pradel, R., and Lebreton, J.-D. 1999. Comparison of different approaches to study the local recruitment of breeders. Bird Study, **46**(Suppl.): S74–S81.

Pradel, R., Johnson, A.R., Viallefont, A., Nager, R.G., and Cezilly, F. 1997. Local recruitment in the greater flamingo: a new approach using capture–mark–recapture data. Ecology, **78**: 1431–1445.

Schwarz, C.J., and Arnason, A.N. 1996. A general methodology for the analysis of open-model capture recapture experiments. Biometrics, **52**: 860–873.

Schwarz, C.J., and Arnason, A.N. 2000. The estimation of age-specific breeding probabilities from capture–recapture studies. Biometrics. In press.

Schwarz, C.J., and Stobo, W.T. 1999. Estimation and effects of tag-misread rates in capture–recapture studies. Can. J. Fish. Aquat. Sci. **56**: 551–559.

Schwarz, C.J., Bailey, R.E, Irvine, J.R., and Dalziel, F.C. 1993. Estimating salmon spawning escapement using capture–recapture methods. Can. J. Fish. Aquat. Sci. **50**: 1181–1191.

Seber, G.A.F. 1965. A note on the multiple recapture census. Biometrika, **52**: 249–259.

Stobo, W.T., and Zwanenburg, K. 1990. Grey seal (*Halichoerus grypus*) pup production on Sable Island and estimates of recent production in the Northwest Atlantic. *In* Population biology of sealworm (*Pseudo terranovea decipiens*) in relation to its intermediate and seal hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222. pp. 171–184.

Zwanenburg, K., and Bowen, W.D. 1990. Population trends of the grey seal (*Halichoerus grypus*) in eastern Canada. *In* Population biology of sealworm (*Pseudo terranovea decipiens*) in relation to its intermediate and seal hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222. pp. 185–197.

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Are all the tables and figures needed and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data depository?

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Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

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Parts of the manuscript

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Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

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Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

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Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

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Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine...we..." If the section is long, consider using subheadings corresponding to headings for the findings.

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Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

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Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

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References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence**. Nearly every manuscript submitted to the *Journal* contains errors in the references.

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Leave three spaces above and below lines that have much superscript material, and above and below equations and formulae. In the various expressions, place each component in its correct position.

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Photographs should be *continuous tone* on glossy paper. Prints must be of high quality, on glossy paper, with strong contrast. The copies for reproduction should only show essential features, be trimmed, and mounted on **thin** flexible white bristol board with no space between those arranged in groups. A photograph, or group of them, should be planned to fit into the area of either one or two columns of text **with no further reduction.** Electron micrographs or photomicrographs should include a scale bar directly on the print. The best results will be obtained if the authors match the contrast and density of all figures arranged as a single plate.

Color illustrations will be at the author's expense. Further details on prices are available from Cecily Pearson, Managing Editor of the *Journal* (613-993-9099; fax: 613-952-7656; e-mail: cecily.pearson@nrc.ca).

NRC Research Press prefers the submission of electronic illustration files for accepted manuscripts and will use these electronic files whenever possible. If electronic files are not available, paper versions of the figures will be scanned. Note that the scanner will easily reproduce flaws (e.g., correction fluid, smudges). Submission of noncontinuous (screened) photographs and scanned illustrations printed out on laser printers is not recommended as moirés develop; a moiré is a noticeable, unwanted pattern generated by rescanning or rescreening an illustration that already contains a dot pattern.

Preparation of electronic illustration files

Authors must supply copies of the original electronic files (i.e., the files directly from the software application in which they were created) and **high-quality laser proofs** of the images. **Electronic files (i.e., figure number and figure content) should match the laser proofs.** On the disk label, identify (1) the software application and version; (2) file name(s), size, and extension; and (3) if the files have been imported from another software. If you have compressed your files, indicate what compression format was used. PC or Macintosh versions of True Type or Type 1 fonts should be used. **Do not use bitmap or nonstandard fonts.** Electronic graphics can be accepted on the following: 3½-in. disks, 100 MB Zip cartridge, and CD-ROM.

All figures should be submitted at their final published size. For figures with several parts (e.g., *a*, *b*, *c*, *d*, etc.) created using the same software application, assemble them into one file rather than sending several files.

Remember that the more complex your artwork becomes, the greater the possibility for problems at output time. Avoid complicated textures and shadings, especially in vector illustration programs; this increases the chance for a poor-quality final product.

Bitmap (raster) files—Bitmaps are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid.

The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art and finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

All *colour* files submitted must be as CMYK (cyan, magenta,

yellow, and black). These colours are used in full-colour commercial printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector files—Vector files are image files produced using elements such as lines and shapes. Typically these files are used for *line drawings*.

Bitmaps inside vector files—Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

The preferred graphic application of NRC Research Press is CorelDraw! For other applications that can be used, see electronic graphics list at http://www.nrc.ca/cisti/journals/elegraph.html.

Format conventions

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel (Bq)
calorie (cal)
centimetre (cm)
centimetre, square (cm^2)
centimetre, cubic (cm^3)
centimetres per gram per second ($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb (C)
decimetre (dm)
degree Celcius (°C)
degrees of freedom (df)
gram (g)
hectare (ha)
hertz (Hz)
hour (h)
joule (J)
kilometre (km)
litre (L)
lumen (lm)
lux (lx)
metre (m)
metre, square (m^2)
metre, cubic (m^3)
micrometre (µm)
milligram (mg)
millilitre (mL)
millimetre (mm)
millimetre, square (mm^2)
minute (min)
molar mass (M)
mole (mol)
moles per litre (mol/L, M)
Pascal (Pa)
second (s)
standard deviation (SD)
standard error (SE)
tonne (metric ton) (t)
volt (V)
volume (vol)
watt (W)

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the...), or the alternative sequence month-day-year may be used with the year offset by commas (On October 9, 1983, the...).

Reference citations in text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared..." or "...were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared..." or "...were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "personal communication."

Unpublished data

If an unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "In press." Only those manuscripts that are in galley or page proof stage or for which there is an acceptance letter can be considered in press. If an article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpublished data" in the text and do not include it in the reference list.

Reference lists

References should be listed at the end of the paper in alphabetical order according to surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *BIOSIS Serial Sources*) for references.

Journal article

Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. **39**: 1444–1452.

Entire issue of journal

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Book in a series

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Book not in a series

LeBlond, P.H., and Mysak, L.A. 1978. Waves in the ocean. Elsevier, New York.

Part of book

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. Neil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 203–229.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Stan-

dard methods for the examination of water and wastewater. 14th ed. Washington, D.C.

Theses

Kutty, M.N. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. No. 1032.

Translation

Koike, A., and Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. (Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used because they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration, whereas 12:30 refers to the time of day.

Word list

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., addition.)

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URL: http://www.nrc.ca/cisti/journals/

Revised January 2000

Recommandations aux auteurs

Journal canadien des sciences halieutiques et aquatiques

La Rédaction encourage la soumission d'articles contribuant à l'enrichissement des connaissances des sciences halieutiques et aquatiques. Les articles peuvent porter sur les cellules, les organismes, les populations, les communautés, les écosystèmes ou les procédés qui affectent les systèmes aquatiques. Ils peuvent traiter de disciplines variées y compris la biologie et l'écologie des organismes d'eau douce et d'eau salée, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, les maladies et la gestion. Les articles sont choisis selon la portée et l'importance des nouvelles connaissances ou idées qu'ils contiennent. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux.

Nous invitons les auteurs à soumettre des articles ayant des objectifs précis ou relevant des hypothèses, concepts ou questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. La raison d'être de l'analyse et l'interprétation des résultats doivent être situées dans un vaste contexte disciplinaire ou interdisciplinaire. Les articles de méthodologie et de modélisation doivent inclure des applications et prouver la supériorité de la méthodologie ou du modèle explicité.

La Rédaction décourage la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicable à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie. Les études préliminaires ou fragmentaires, ou dont la pertinence à grande échelle n'est pas démontrée, et les interprétations purement spéculatives sont carrément rejetées.

Les articles doivent être aussi complets que possible. S'il est impossible d'inclure toute les données pertinentes dans un seul article, l'auteur devra inclure des renvois à des articles connexes et soumettre ceux-ci avec son propre article.

Le *Journal* publie le genre de textes suivants :

Articles—Études de vaste portée qui constituent de nouvelles contributions à la science.

Perspectives—Synthèses, critiques ou réévaluations de concepts ou de paradigmes couramment admis.

Discussions—Commentaire ou réponse sur des sujets récemment abordés dans le *Journal*.

Communications rapides—Présentations de nouveaux concepts, descriptions de nouvelles méthodologies ou courts textes sur des sujets d'actualité ou controversés.

On peut obtenir les caractéristiques précises de chacun de ces genres sur le site web du Bureau de la rédaction (http://www.uoguelph.ca/~cjfas).

Processus de publication

Les manuscrits (**en triple**) doivent être soumis aux directeurs scientifiques, *Journal canadien des sciences halieutiques et aquatiques*, University of Guelph, Department of Zoology, Building #006, Room 107, Guelph, ON N1G 2W1, Canada, et accompagnés d'une lettre qui fait état (1) des principaux points et de l'importance du travail, (2) de la participation de tous les auteurs au manuscrit et de leur approbation de ce dernier, (3) de l'assurance que le manuscrit en question n'est pas en cours d'examen aux fins de publication dans une autre revue, (4) de suggestions quant aux arbitres éventuels, (5) des autres manuscrits, y compris de résumés étoffes contenant une information identique ou similaire à celle contenue dans le manuscrit soumis et (6) des numéros de téléphone et de télécopieur ainsi que de l'adresse électronique et de l'adresse au courant de chaque auteur. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les demi-teintes) jusqu'à ce qu'on les lui demande. Veuillez inclure le texte, les tableaux et les figures du manuscrit sur disquette, en précisant le(s) progiciel(s) et la (les) version(s) utilisés; le logiciel de traitement de textes préférés est Microsoft Word pour **l'examen par les réviseurs. Les manuscrits et les illustrations doivent être solidement emballés afin d'être protégés lors de l'expédition.**

Chaque article est habituellement soumis à une évaluation par au moins deux réviseurs avant d'être examiné par les directeurs scientifiques ou les directeurs scientifiques associés. Toutefois, les directeurs scientifiques renverront sans les évaluer tout article qui ne reflète pas la portée ou le caractère du *Journal* et les articles qui débordent des limites imposées pour la publication sous forme de « résumés approfondis ». On obtiendra les directives à cet égard sur le site web du Bureau de la rédaction (http:\\www.uoguelph.ca/~cjfas). Les articles soumis aux fins de publication dans un supplément sont soumis au même rigoureux exercice d'examen que les articles des numéros réguliers.

Voici quelques recommandations générales. Les articles ne doivent pas compter plus de 36 pages dactylographiées à double interligne, y compris les références, les tableaux et les figures. L'auteur devrait limiter le nombre de références à 36 et le nombre combiné de tableaux et d'illustrations à 12. Nous encourageons la soumission d'articles brefs, de style clair. Nous accepterons d'examiner les articles plus longs (p. ex. comptant plus de tableaux et de figures que la norme) et les perspectives intensément étayées (c.-à-d. comptant beaucoup de références) pour autant que leur importance et leur interprétation de nouvelles connaissances en justifient la longueur.

On demande habituellement aux auteurs d'examiner les commentaires des réviseurs et de modifier leur texte en conséquence en 90 jours, tout au plus. Faute de respecter ce délai, l'article renvoyé par l'auteur sera traité comme une nouvelle

Also available in English

soumission à moins que l'auteur n'ait au préalable dûment communiqué avec le Bureau de la rédaction.

Droits de reproduction—Lorsqu'un article contient de la documentation (tableaux, figures, schémas, etc.) déjà publiée, il incombe à l'auteur d'obtenir **une permission écrite du titulaire de ces droits d'auteur** pour permettre la reproduction de l'information **en format imprimé et électronique.** La lettre d'autorisation doit accompagner le manuscrit soumis, sans quoi le processus de publication pourra être retardé. Cette lettre sera exigée avec toute mention du type « Tirée de... ». Si une illustration est modifiée, de sorte qu'une telle autorisation n'est pas requise, la formulation doit être explicite : « Modifiée d'après... ». Si l'illustration est reconstitutée à partir d'informations tirées d'autres auteurs, la mention doit être du même type que pour une référence.

Soumission de la version approuvée—Les auteurs sont priés de soumettre **une copie imprimée ainsi qu'une copie sur disquette de la version approuvée de l'article**. Les fichiers de texte et les fichiers d'illustrations devront être soumis sur des disquettes distinctes. Le nom de l'auteur doit être indiqué clairement sur toute disquette soumise. Le **texte** (y compris les tableaux) devrait être soumis en format de traitement de texte (WordPerfect ou Microsoft Word est préférable, compatible à l'IBM ou Macintosh) **et** en fichier ASCII. Il importe d'indiquer le logiciel de traitement de texte, la version, de même que le type d'ordinateur (IBM ou Macintosh). En ce qui a trait aux **illustrations**, voir la section « Préparation des fichiers électronique des illustrations ». Tout article devra être accompagné d'une lettre faisant foi que la version du texte sur disquette reflète **exactement** la version imprimée soumise.

Épreuves en placard — Une épreuve en placard, des épreuves des illustrations, une copie de l'article corrigé et un bon de commande de tirés à part sont envoyés à l'auteur correspondant. **Les épreuves en placard doivent être relues attentivement car elles ne sont pas corrigées par les Presses scientifiques du CNRC**. Elles doivent en outre être retournées dans les 48 heurs suivant leur réception. Au stade des épreuves, il n'est plus temps d'apporter des modifications importantes ni de faire des ajouts ou des suppressions. Les frais occasionnés par des modifications excessives devront être assumés par les auteurs.

Tirés à part — Pour obtenir des tirés à part, il est nécessaire de remplir le bon de commande à cet effet et de le retourner avec le paiement (chèque, numéro de carte de crédit, numéro du bon d'achat ou pièce de journal interne) en même temps que les épreuves et l'article corrigés. Le tarif des tirés à part commandés après la parution du numéro du *Journal* est fortement majoré. **Le *Journal* n'offre pas de tirés à part gratuits; ceux-ci ne sont postés qu'après réception d'un numéro du bon d'achat ou du paiement.**

Transfert des droits d'auteur—Tous les auteurs doivent compléter un formulaire de transfert des droits d'auteur au CNRC. On obtiendra ces formulaires auprès du directeur scientifique, dans tout récent numéro du Journal ou sur le site web des Presses scientifiques du CNRC (http://www.nrc.ca/cisti/journals/coptransf.html).

Toute permission de republication partielle ou intégrale d'un article doit être obtenue auprès des Presses scientifiques du CNRC.

Préparation des manuscrits

Directives générales

Le processus de publication sera grandement facilité si les articles sont préparés conformément au style du *Journal*.

Dactylographiez le manuscrit sur papier blanc (8,5 sur 11 po) sur un côté de la page. Laissez toujours une marge d'au moins 1 po. Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations. Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à **double interligne**. Si on ne dispose pas d'une police d'italique, souligner les passages à imprimer en italique. En aucun endroit du manuscrit n'écrivez entièrement en majuscules. Pour les références citées dans le texte, utilisez le système nom et année.

Dispositions générales

Le *Council of Biology Editors Style Manual* (6^{e} éd., 1994, Council of Biology Editors, Inc., Chicago, IL 60603, U.S.A.) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant *BIOSIS Serial Sources* (BIOSIS, 2100 Arch Street, Philadelphia, PA 19103-1399, U.S.A.). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5^{e} éd., 1991. Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee et al. (1990. Trans. Am. Fish. Soc. **119**: 2–15). Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionées dans un éditorial du *Journal*, vol. 40, No. 12), et se conformer au *Guide canadian de familiarisation au système métrique* CAN3-Z234.1–79 (l'Association canadienne de normalisation, 178, boulevard Rexdale, Rexdale, ON M9W 1R3, Canada).

Liste de contrôle pour manusrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs :

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données?

Si une analyse statistique est effectuée, la statistique cède-t-elle le pas à la recherche? Dans des énoncés de probabilité, les tableaux statistiques inutiles sont-ils exclus et seulement les tests statistiques cités?

Le texte gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il représentatif du contenu du rapport? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème? La section *matériel et méthodes* contient-elle le minimum de détails qui permettront aux lecteurs de saisir les grandes lignes de l'étude et de juger de la valeur des données? Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et

déductions? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Sont énumérés, ci-dessous, des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

(1) Limiter le résumé à un paragraphe d'environ 175 mots.
(2) Dactylographier à double interligne tous les éléments du manuscrit, y compris la bibliographie et les légendes des tableaux et des figures.
(3) Éviter partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.
(4) Ne composer en italique que les noms latins d'organismes et certaines notations statistiques et mathématiques qui devront être imprimés en italique.
(5) Dans les figures, les tableaux et le texte, exprimer les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placer un zéro avant la virgule décimale.
(6) Éviter les formes ambiguës, telles que g C/m^2/jour; utiliser plutôt g C·m^{-2}·$jour^{-1}$.
(7) Identifier le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.
(8) Dans le texte, généraliser à partir des tableaux et des figures; éviter une répétition de tous les détails. S'assurer que tous les tableaux et figures sont indépendants et mentionnés dans le texte dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.
(9) Préparer la section des remerciements au stade du manuscrit et non à celui des épreuves.
(10) En référant à des communications personnelles, donner les initiales et l'adresse postale de la personne en cause.
(11) Éliminer toutes références qui ne s'appliquent pas directement au problème.
(12) Vérifier soigneusement la correspondance des références en regard des citations dans le texte et vice versa. Dans le cas de matériel difficilement accessible, indiquer l'endroit où le trouver (p. ex., disponible auprès du Département de biologie, Université Laval, Sainte-Foy, QC G1K 7P4, Canada).
(13) Éliminer les virgules entre le nom et la date dans les citations telles que (Smith 1990) et ne pas souligner ni mettre en italique « et al. »
(14) Dans les en-têtes de tableaux et sur les axes des figures, remplacer 10^n par les préfixes SI appropriés ou rédiger en toutes lettres (ex., milliers).
(15) Aux fins de la révision, les photocopies des figures, sauf pour les demi-teintes, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyer des photographies, des imprimés laser, ou encore les dessins originaux/fichiers électroniques (voir « Illustrations » et « Préparation des fichiers électroniques des illustrations »). Utiliser autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organiser le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dresser une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retoucher les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si méthodes, résultats et discussion sont décrits dans des sections séparées.

Organiser les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. S'assurer que ces derniers sont aussi clairs que possible sans référence au texte.

Commencer les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si la conclusion est donnée d'abord puis appuyée ensuite, non seulement le travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voir à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composer la phrase thème de chaque paragraphe et disposer ces phrases dans l'ordre approprié.

Titre

Limiter le titre à ce que l'étude veut démontrer. C'est la clé de l'article et il devrait refléter clairement et brièvement le contenu de l'étude. Le titre a deux fonctions : (1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et (2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance potentielle de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex., rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à... » ou « Recherche sur... ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires. On évitera les titres en séries.

Résumé

Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquer, au début du résumé, la principale conclusion de l'étude et l'appuyer par des découvertes pertinentes. Ne donner que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et les insérer dans l'énoncé des découvertes. Éviter l'emploi de phrases telles que «... on y analyse » ou «... a été trouvé » ; être plus spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir; il importe donc qu'il reflète précisément le contenu du travail et qu'il soit complet en lui-même (c.-à-d. les références absolument *essentielles* sont indiquées, sous forme retraçable, p. ex., R.B. Deriso. 1980. J. Can. Sci. Halieut. Aquat. **37** : 268–282).

Introduction

Limiter l'introduction à la portée, au but et à la raison de l'étude. Ne mentionner que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspec-

tive du travail. Commencer avec le but ou la portée du travail, définir ensuite le problème et ajouter ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limiter les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si toute l'information est présentée, autant que possible, dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référer aux publications et ne décrire en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer... nous... ». Si la section est longue, utiliser des sous-titres correspondant à ceux de la section des résultats.

Résultats

Limiter les résultats aux réponses apportées aux questions en rapport avec le but de l'étude; que ces réponses soient aussi complètes que possible, tout en étant concises. Exprimer, autant que possible, les découvertes dans les mêmes termes que les observations ou les mesures, évitant ainsi de confondre faits et spéculations. Dégager les généralités des tableaux et des figures et y référer en évitant de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries de rapports ou dans un dépôt de données reconnu; et on y référera dans le texte.

Discussion

Limiter la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, les noter et faire ressortir la manière dont les résultats corroborent ou contredisent les travaux déjà publiés. Limiter la spéculation à ce qui peut être appuyé sur des preuves raisonnables. Terminer cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Éviter de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. **On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa.** Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible. Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½ ou 2 colonnes du *Journal*. Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes. Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires. Chaque tableau doit avoir une légende instructive, complète et indépendante. Les renvois des tableaux doivent être indiqués par des minuscules suscrites dactylographiées au bas du tableau. Placez les tableaux après la liste des références. Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Expressions, équations et formules mathématiques

Laisser trois espaces au-dessus et au-dessous des lignes portant des lettres suscrits ou souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placer chaque composante des différentes expressions dans la position qui lui est propre.

Indiquer clairement tous les symboles. Épeler les lettres grecques en marge, à la première mention. Faire une distinction entre la lettre *l* et le chiffre *un*, et entre la lettre majuscule *O* et le chiffre *zéro*. Insérer de la même façon toute autre note explicative.

Annexes

Les illustration et les tableaux en annexe doivent être numérotés différemment des illustrations et tableaux du corps de l'article, p. ex. fig. A1, tableau A1, etc.

Documentation complémentaire

La documentation complémentaire, telle que les grands tableaux et les données et les cartes détaillées qui ne sont pas essentiels à la compréhension du document, peut être versée, à la demande des auteurs ou des directeurs scientifiques, au Dépôt des données non publiées, ICIST, Conseil national de recherches du Canada, Ottawa, ON K1A 0S2, Canada; des copies peuvent être obtenues contre paiement. Les auteurs qui désirent utiliser ce service doivent indiquer les parties dont ils demandent le dépôt. Toute donnée déposée fait l'objet d'une note infrapaginale à la section appropriée du document. Les tableaux et les figures doivent être numérotées de façon consécutive, distinctement de ceux destinés à la publication (p. ex. : figure D1, tableau D1).

Illustrations

Fournir des photographies, des imprimés laser (les imprimés par imprimante par points sont inacceptables) ou les dessins originaux (pourvu qu'ils ne dépassent pas 8,5 sur 11 po) de chaque illustration. Transmettre trois copies des illustrations qui serviront au moment de la révision.

Chaque figure ou groupe de figures doit être préparé de manière à occuper, après une réduction appropriée, l'espace d'une ou deux colonnes du texte. Les dimensions finies maximales d'une illustration sur une colonne sont de 8,8 sur 23,9 cm (3,5 sur 9,4 po) et celles d'une illustration sur deux colonnes est de 18,2 sur 23,9 cm (7,2 sur 9,4 po). Les figures (y compris les similigravures) sont numérotées en chiffres arabes selon leur ordre séquentiel et doivent être annoncées dans le texte bien que tous leurs éléments doivent être intelligibles en eux-mêmes.

Tous les termes, les abréviations et les symboles qui se trouvent dans les figures doivent être les mêmes que ceux apparaissant dans le texte. Seuls les détails essentiels devraient être identifiés; l'information plus détaillée devrait paraître dans la légende. Chaque illustration doit être identifiée, de préférence au bas, à gauche, par le numéro de la figure et les noms des auteurs, suffisamment éloignés de l'espace réservé à l'illustration.

Les **dessins au trait** originaux doivent être en encre de Chine noire ou produits par ordinateur en encre noire sur du papier blanc de première qualité ou un matériau comparable. Quant aux graphiques produits par ordinateur, il faut fournir un imprimé au laser de la meilleure résolution disponible. **Les photocopies ne sont pas acceptables.**

L'épaisseur des lignes doit être suffisante (au moins 0,5 point) pour que la reproduction soit nette; tous les symboles, les indices supérieurs ou inférieurs et les signes décimaux doivent être en bonne proportion avec le reste du dessin et suffisamment marqués pour permettre la réduction nécessaire sans perte de détail. Ne pas utiliser de petits symboles ouverts qui ont tendance à se refermer lorsqu'ils sont reproduits. **Le lettrage produit par imprimante à matrice de point ou par machine à écrire, ou à la main, ne sont pas acceptables.** Le même style de police et la même dimension de lettrage doivent servir dans toutes les figures de dimensions semblables d'un article.

Les **cartes** doivent avoir des motifs clairs et gras, et comporter les longitudes et latitudes (ou les coordonnées de Mercator transverses universelles) de même qu'une échelle graphique. Tous les noms d'endroits et les caractéristiques géographiques sur les cartes du Québec doivent être en français seulement, avec les accents et les majuscules appropriés.

Les **photographies** doivent être en demi-teintes sur papier glacé. Les épreuves doivent être de première qualité, sur papier glacé, avec contrastes bien marqués. Les copies destinées à la reprographie doivent être rognées de façon à ne laisser paraître que les éléments essentiels et être montées sur carton bristol blanc **léger** et flexible. Aucun espace ne doit paraître entre les photographies constituant une même planche. Une photographie ou un groupe de photographies doit être préparé de manière à occuper, **sans réduction supplémentaire**, une ou deux colonnes du texte. Les micrographies électroniques et les photomicrographies doivent comporter une échelle graphique à même l'illustration. On évitera les pertes de détails et de contraste inhérentes au procédé d'imprimerie en agençant le contraste et la densité de toutes les photographies montées sur une même planche.

Les **illustration en couleurs** sont aux frais de l'auteur. On peut obtenir de plus amples renseignements sur les prix auprès de Cecily Pearson, rédactrice administrative du *Journal* (613-993-9099; télécopieur : 613-952-7656; adresse électronique : cecily.pearson@nrc.ca).

Les Presses scientifiques du CNRC préfèrent recevoir la version électronique des illustrations des manuscrits acceptés et l'utiliseront dans la mesure du possible. Si les fichiers électroniques ne sont pas disponibles, la version papier des illustrations sera balayée. Toutefois, le lecteur optique reproduit facilement les défauts (p. ex., le liquide correcteur, les maculations). Il est recommandé de ne pas soumettre de photographies tramées, ni des illustrations balayées imprimées au laser, à cause des moirures qui se forment; une moirure est un motif perceptible et non désiré produit par le rebalayage ou le retramage d'une illustration qui contient déjà un motif de points.

Préparation des fichiers électroniques des illustrations

Les auteurs doivent fournir des copies des fichiers électroniques originaux (c.-à-d. les fichiers tirés directement du logiciel sur lequel ils ont été créés) et des **épreuves au laser de haute qualité** des images. **Les fichiers électroniques (c-à-d. le numéro et le contenu de la figure) doivent correspondre aux épreuves au laser**. Sur l'étiquette de la disquette, il faut indiquer (1) l'application et la version du logiciel; (2) le(s) noms(s), la dimension et le suffixe du fichier; et (3) si les fichiers ont été importés d'un autre logiciel. Si les fichiers ont été comprimés, il faut indiquer le format de compression. Les versions PC ou Macintosh des polices True Type ou Type 1 doivent être utilisées. **On ne doit pas se servir des polices de caractères en mode points ou non standard.** Les graphiques électroniques sont acceptés sur les disquettes suivantes : disquettes 3½ po, cartouche Zip de 100 Mo et sur CD-ROM.

Toutes les illustrations doivent être soumises dans leur dimension de tirage final. Quant aux figures comportant plusieurs parties (p. ex., *a*, *b*, *c*, *d*, etc.) crées sur le même logiciel, elles doivent être assemblées dans un seul fichier plutôt que de transmettre plusieurs fichiers.

Il faut **garder** les illustrations le plus simple possible afin d'éviter les embûches. Éviter les textures et les fonds grisés compliqués, particulièrement dans les programmes d'illustration vectorielle.

Fichier en mode point—Les fichiers d'images en mode point sont produits sur une grille dans laquelle chaque carré (ou pixel) correspond à une nuance de noir, de couleur ou de gris. Un fichier en mode point est défini par le nombre de pixels ou d'éléments par pouce. Comme le pixel est défini comme un point, on parle aussi de « points par pouce » ou « ppp ». Plus la résolution de l'image est grande, plus le nombre de points sur une surface donnée est élevé.

Les illustrations soumises en mode point doivent être de définition adéquate. Les exigences minimales de définition sont les suivantes : 600 ppp pour les dessins au trait et les lignes fines (les dessins au trait comportant des lignes fines ou un fond grisé), 300 ppp pour les demi-teintes et les couleurs et 600 ppp pour les combinaisons (demi-teintes avec lettrage à l'extérieur de l'espace réservé à la photo).

Tous les fichiers en *couleurs* soumis doivent suivre la base CMJK (cyan, magenta, jaune et noir). Ces couleurs sont utilisées dans l'impression commerciale tout en couleur. Les graphiques RVB (le rouge, le vert et le bleu sont les couleurs utilisées particulièrement pour produire les images sur un écran) ne s'impriment pas correctement.

Fichiers vectoriels—Les fichiers vectoriels sont des fichiers d'images composées d'éléments comme les lignes et les formes. Ce type de fichier convient habituellement aux *dessins au trait.*

Fichiers vectoriels en mode point—Le mode points peut être importé dans un logiciel vectoriel ou de dessins aux seules fins d'ajouter et de superposer de l'information, des lignes, du texte, etc. Le mode points ne doit pas être redimensionné, recadré, tourné ou manipulé de toute autre façon après avoir été importé.

Le logiciel graphique préféré par les Presses scientifiques du CNRC est CorelDraw! Pour les autres applications possibles, reportez-vous à la liste des programmes graphiques électroniques à http://www.nrc.ca/cisti/journals/elegrapf.html.

Conventions

Abréviations

N'abréger les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

becquerel (Bq)
calorie (cal)
centimètre (cm)
centimètre carré (cm^2)
centimètre cube (cm^3)

centimètres par gramme par seconde ($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb (C)
décimètre (dm)
degré Celsius (°C)
degrés de liberté (dl)
écart type (ET)
électronvolt (eV)
erreur type (ErT)
farad (F)
gramme (g)
hectare (ha)
hertz (Hz)
heure (h)
joule (J)
kilomètre (km)
litre (L)
lumen (lm)
lux (lx)
mètre (m)
mètre carré (m^2)
mètre cube (m^3)
micromètre (µm)
milligramme (mg)
millilitre (mL)
millimètre (mm)
millimètre carré (mm^2)
minute (min)
masse molaire (M)
mole (mol)
moles par litre (mol/L, M)
pascal (Pa)
seconde (s)
tonne (métrique) (t)
volt (V)
volume (vol)
watt (W)

Dates

On écrira les dates dans l'ordre jour-mois-année, p. ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié: « Brown (1983) compare... » ou «...on a comparé (Brown 1983) ». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « et al. » : « Brown et al. (1983) comparent... » ou «...on a comparé (Brown et al. 1983) ».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « communication personnelle ».

Données inédites

Si un livre ou un article inédit a été accepté pour publication, insérez-le dans la liste des références, avec la mention « Sous presse ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si un article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

Les références doivent être classées par ordre alphabétique des noms des premiers auteurs. Si la liste comprend plusieurs publications d'un même premier auteur, les règles suivantes seront appliquées. (1) S'il n'y a qu'**un auteur**, les ouvrages se succèdent par ordre chronologique de partition en commençant par le plus ancient. Si l'auteur a publié, au cours d'une année, plusieurs livres ou articles, on distinguera les références les unes des autres en faisant suivre l'année de publication d'une lettre (*a, b, c,* etc.). (2) S'il y a **deux auteurs**, les ouvrages sont placés à la suite de ceux n'ayant qu'un auteur, et sont alors classés par ordre alphabétique du nom du deuxième auteur. (3) S'il y a **trois auteurs ou plus**, les ouvrages sont placés à la suite de ceux ayant deux auteurs et sont classés par ordre chronologique.

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après *CASSI* ou *BIOSIS Serial Sources*).

Article d'une revue

Archambault, D., et Bourget, E. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctiques. J. Can. Sci. Halieut. Aquat. **40** : 1278–1292.

Numéro complet d'une revue

Gordon, D.C., Jr., et Hourston, A.S. (*Éditeurs*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. J. Can. Sci. Halieut. Aquat. **40**(suppl. 1).

Livre dans une série

Hilton, J.W., et Slinger, S.J. 1981. Nutrition et alimentation de la truite arc-en-ciel. J. Can. Sci. Halieut. Aquat. publ. spéc. n° 55.

Livre ne faisant pas partie d'une série

Forrester, W.D. 1983. Manuel canadien des marées. Service hydrographique du Canada, ministère des Pêches et des Océans, Ottawa.

Partie d'un livre

Aubert, M., Aubert, J., Daniel, S., et Desirotte, N. 1982. État actuel de la pollution bactérienne au large des côtes françaises. *Dans* La pollution des mers et les ressources biologiques. *Éditeur* : M. Ruivo. Fishing News (Books) Ltd., London. pp. 36-40.

Corporation auteur

American Public Health Association, American Water Works Association et Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14e éd. Washington.

Thèse

Delisle, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse de doctorat, Université d'Ottawa, Ottawa.

Rapport

Dutil, D.J., Fortin, M., et Vigneault, Y. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. Manuscr. Can. Sci. Halieut. Aquat. n° 1653.

Traduction

Koike, A., et Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64** : 1–11. (Traduit du japonais par Can. Transl. Fish. Aquat. Sci. 4950, 1983.)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles « h, » « min » et « s » ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12 : 30 rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, « cold water » (n.) «cold-water » (adj.); « hard water » (n.) « hardwater » (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

Presses scientifiques du CNRC
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